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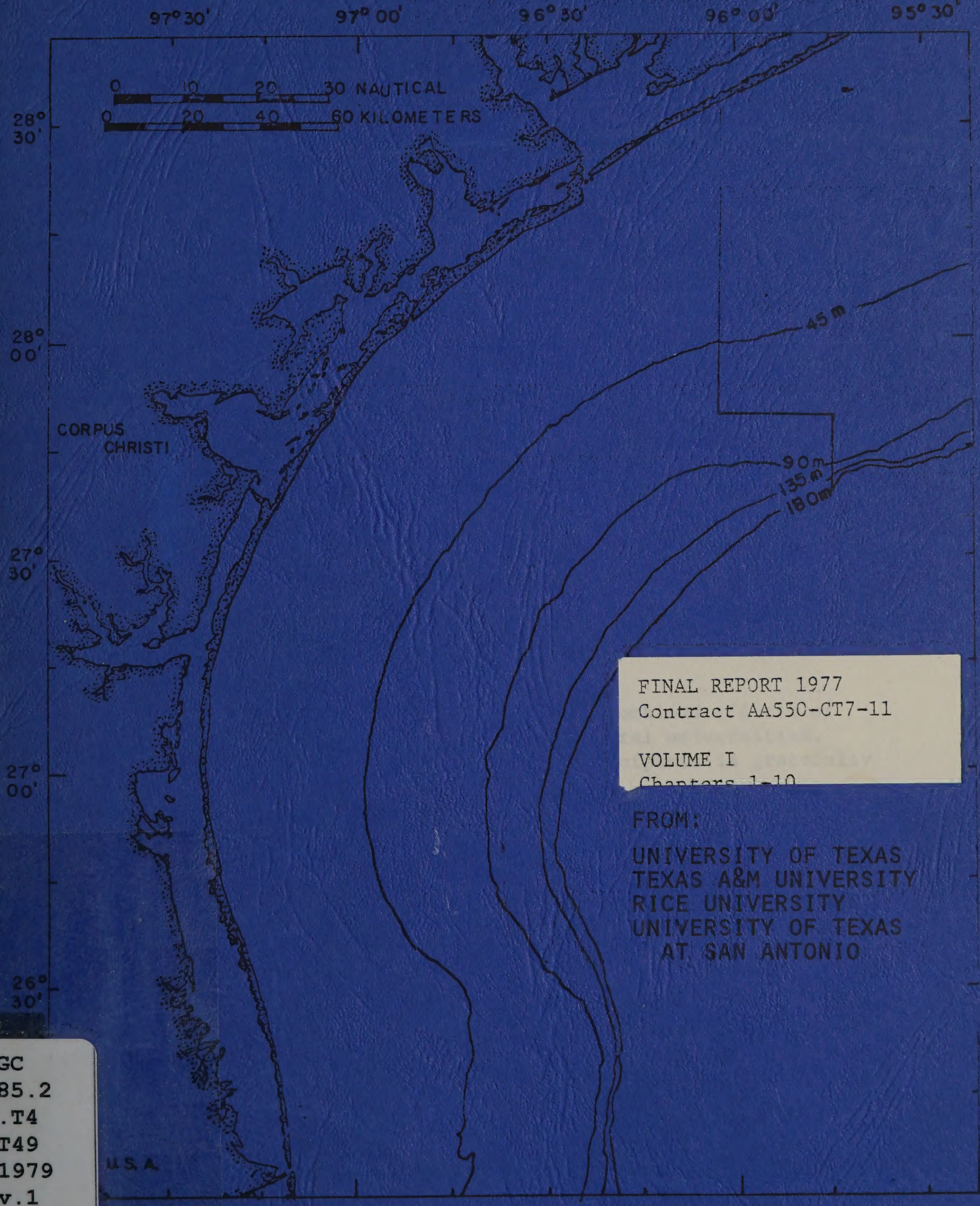

FINAL REPORT 1977
Contract AA550-CT7-11

VOLUME I
Chapters 1-10

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ENVIRONMENTAL STUDIES,
SOUTH TEXAS OUTER CONTINENTAL SHELF,
BIOLOGY AND CHEMISTRY

Submitted to:

The Bureau of Land Management
Washington, D. C.

by

The University of Texas Marine Science Institute
Port Aransas Marine Laboratory
Port Aransas, Texas 78373

Acting for and on behalf of
a consortium program conducted by:

Rice University
Texas A&M University
University of Texas

FINAL REPORT 1977
Contract AA550-CT7-11

VOLUME I
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January 15, 1979

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FOREWORD

This study of the South Texas Outer Continental Shelf (STOCS) was conducted on behalf of the U.S. Bureau of Land Management and with the close cooperation of personnel of that agency. The studies reported on herein constituted the third year of a sampling program of chemical and biological parameters of the STOCS. This study was part of an overall program of the STOCS, other elements being (1) geology and geophysics by the U.S. Geological Survey, (2) fisheries resources and ichthyoplankton populations by the National Oceanographic and Atmospheric Administration/National Marine Fisheries Services, and (3) biological and chemical characteristics of selected topographic features in the Northern Gulf of Mexico by Texas A&M University. The resultant data from this investigation represent the first step in understanding how to assess and control the impact of petroleum exploration and development in the STOCS area. The central goal of these and other environmental quality surveys of continental shelf areas is the protection of the living marine resources from deleterious effects.

This investigation was the result of the combined efforts of scientists and support personnel from several universities. The hard work and cooperation of all participants is gratefully acknowledged.

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CHAPTER ONE

INTRODUCTION

Purpose and Scope of Study

In 1974, the Bureau of Land Management (BLM), as manager of the Outer Continental Shelf Leasing Program, was authorized to initiate a National Outer Continental Shelf Environmental Studies Program. The broad objectives of this program, as stated by the BLM, are:

(a) to provide information about the Outer Continental Shelf (OCS) environment to enable the Department of the Interior to make management decisions regarding OCS oil and gas development; and

(b) to fill environmental information needs of management, regulatory and advisory agencies, both Federal and State, for a broad range of OCS activities, including the preparation and review of environmental impact statements under the National Environmental Policy Act (NEPA) of 1969, issuance of regulations and permits, and implementation of certain other laws, such as the OCS Lands Act, Fish and Wildlife Coordination Act, the Coastal Zone Management Act, and counterpart state laws.

The National Outer Continental Shelf Environmental Studies Program consists of three basic elements: 1) characterization studies, which are conducted during the pre-development period; (2) long-term monitoring studies; and, (3) special studies, which may occur during the characterization and monitoring studies phase. The four major objectives of characterization studies of OCS oil and gas development areas are to:

(a) provide a description of the physical, chemical, geological and biological components, and their interactions, against which subsequent changes or impacts can be compared;

(b) provide information for predicting the effects of OCS oil and gas development activities upon the components of the ecosystem;

(c) identify critical parameters that should be incorporated into a monitoring program; and,

(d) identify and conduct experimental and other special studies as required to meet the basic study objectives.

To accomplish these objectives for the South Texas Outer Continental Shelf (STOCS), the BLM developed the *Marine Environmental Study Plan for the South Texas Outer Continental Shelf*. This plan called for an initial three-year period of intensive study to characterize the physical, chemical, geological and biological components of the OCS marine ecosystem. After evaluation of the initial study results, specific problem-oriented research will become the major focus of future OCS study objectives.

In addition to the biological and chemical components of this program reported on here, two other major field programs were conducted concurrently. The U.S. Geological Survey conducted a program designed to investigate suspended sediments flux, normal and storm transport and deposition of sediments, and sediment geochemistry in the STOCS area. The National Oceanic and Atmospheric Administration/National Marine Fisheries Service conducted studies to investigate the historical distribution and abundance of ichthyoplankton in the area, to elucidate the snapper and grouper fisheries resources, and to determine the magnitude and economic significance of the recreational and associated "commercial/recreational" fisheries in the area. In addition to the above studies restricted to the STOCS study area, Texas A&M University is conducting a major field survey of the biological and chemical characteristics of selected topographic features in the northern Gulf.

Description of the Study Area

Biological Setting

The Texas coastal area is biologically and chemically a two-part marine system, the coastal estuaries and the broad continental shelf. These two components of the marine systems are separated by barrier islands and connected by inlets or passes. The area is rich in finfish and crustaceans, many of which are commercially and recreationally important. Many of the finfish and decapod crustaceans of the STOCS area exhibit a marine-estuarine dependent life cycle, *i.e.* spawning offshore, migrating shoreward as larvae and postlarvae, and utilizing the estuaries as nursery grounds (Galtsoff, 1954; Gunter, 1945). The broad continental shelf supports a valuable shrimp fishery which, as a living resource, contributes significantly to the local economy. An excellent overview of the zoogeography of the northwestern Gulf of Mexico is provided by Hedgepeth (1953).

Location and Bathymetry

The STOCS study area corresponds to the area outlined by the Department of the Interior for oil and gas leasing. The area covers approximately 19,250 km^2 and is bounded by 96°W longitude on the east, the Texas coastline on the west, and the Mexico-United States international border on the south (Figure 1.1). The bathymetry of the STOCS area is shown in Figure 2.1. The continental shelf off south Texas has an average width of about 88.5 km and a relatively gentle seaward gradient that averages 2.3 m/km.

Work Plan

Objectives

The broad objectives of the 1977 study effort of the STOCS area was to continue describing the natural temporal and spatial variability of all major components of the OCS marine ecosystem. It was assumed that

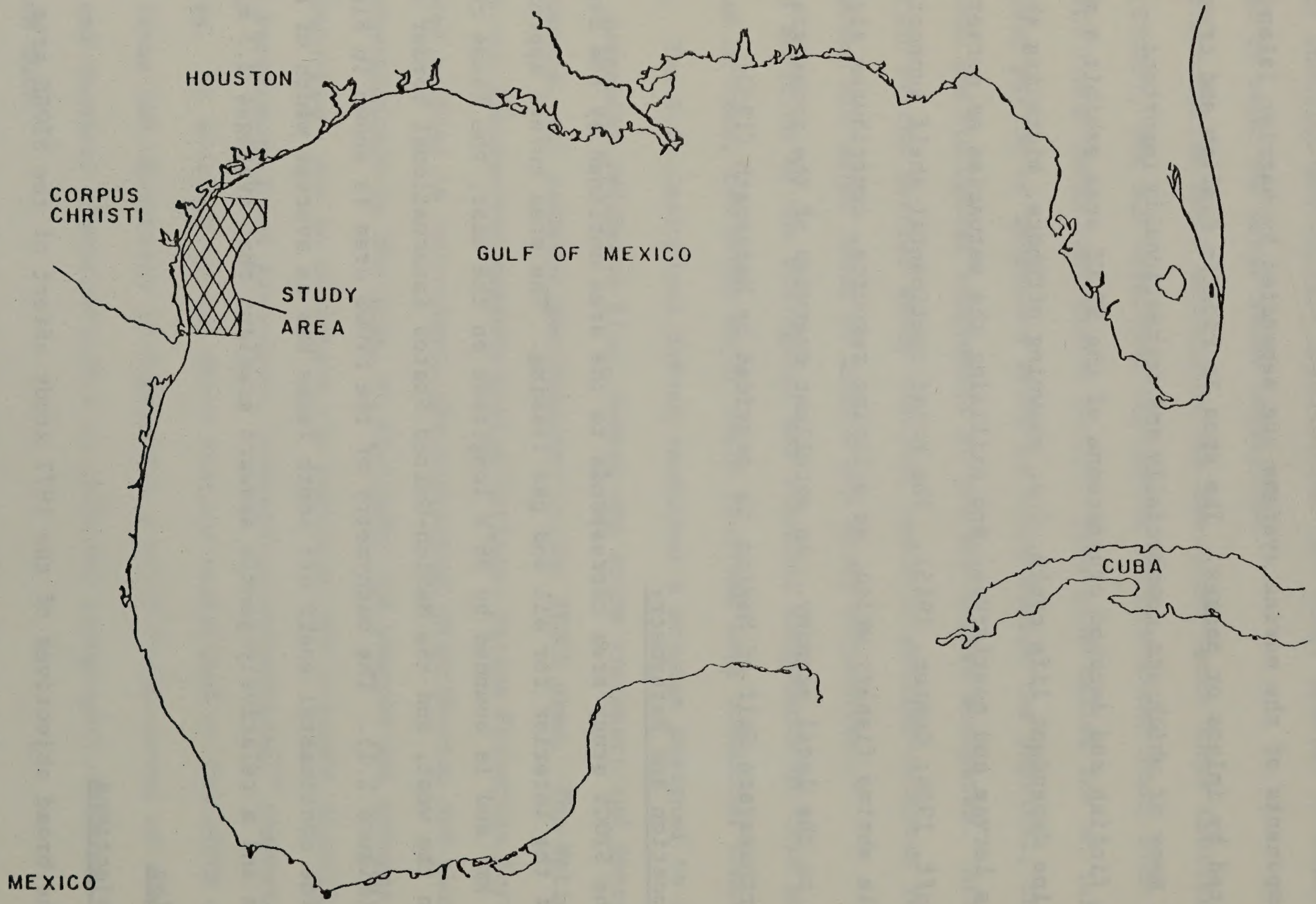

Figure 1.1 Location of the Study Area in Relation to the Entire Gulf of Mexico.

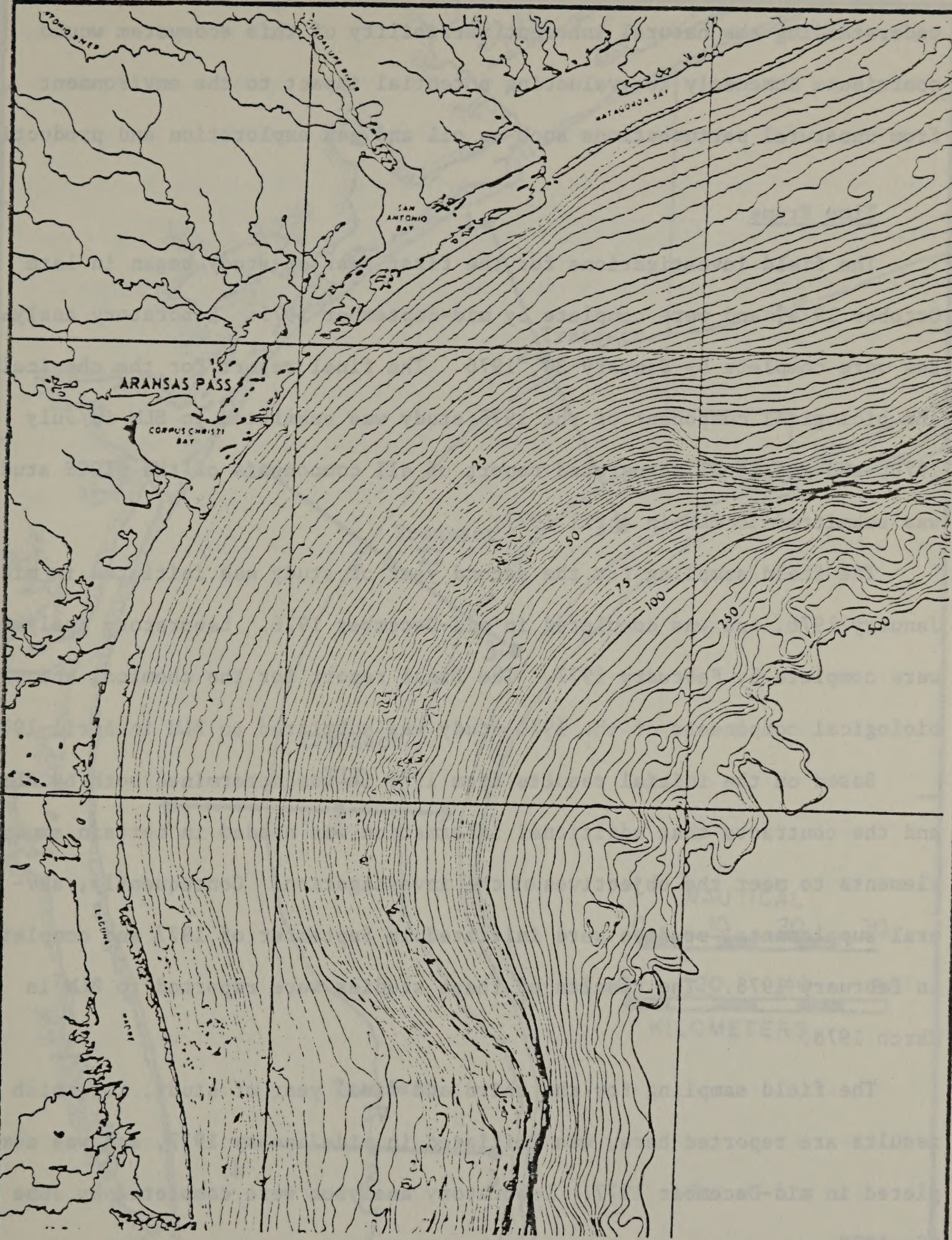

Figure 1.2 Bathymetry of the South Texas Outer Continental Shelf (Depth in Fathoms) From Berryhill *et al.*, 1976, Part I, Figure 3.

understanding the natural inherent varaibility of this ecosystem would contribute immensely to evaluating potential impact to the environment from unnatural perturbations such as oil and gas exploration and production.

Time Frame

The field investigations for the first year of study began in late October 1974, and were complete by mid-September 1975. Laboratory analyses were complete by January 30, 1976. The final report for the chemical and biological component of the 1975 study was submitted to BLM in July 1976, and the final integrated report of all components of the STOCS study was submitted to BLM in April 1977.

The field sampling for the second year of study was initiated in mid-January 1976, and was completed in mid-December 1976. Laboratory analyses were complete by February 1977. The final report for the chemical and biological components of the 1976 study was submitted to BLM in April 1978.

Based on the initial results from 1976 it was determined both by BLM and the contracts that additional information was needed in certain study elements to meet the objectives of the investigation. Consequently, several supplemental studies were initiated in September of 1977 and completed in February 1978. The results of these studies were reported to BLM in March 1978.

The field sampling for the third and final year of study, for which results are reported here, was initiated in mid-January 1977, and was completed in mid-December 1977. Laboratory analyses were completed by June 15, 1978.

Sampling Stations

During the first year of study (1975), twelve stations on four transects were sampled (Figure 1.3). Thirteen additional transect stations

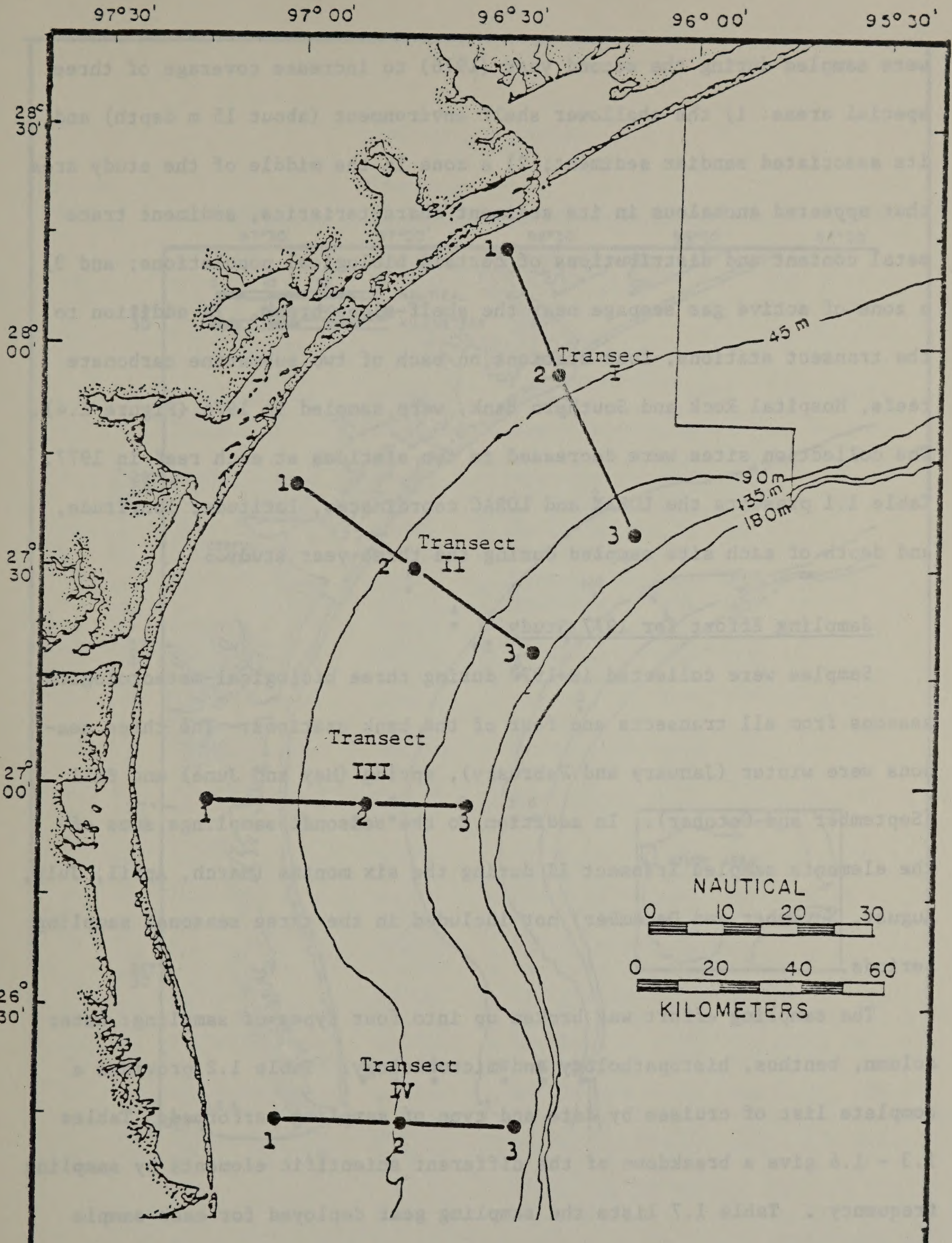

Figure 1.3 Station Locations for the 1975 South Texas Outer Continental Shelf Study. Depth in Meters.

were sampled during the second year (1976) to increase coverage of three special areas: 1) the shallower shelf environment (about 15 m depth) and its associated sandier sediment; 2) a zone in the middle of the study area that appeared anomalous in its sediment characteristics, sediment trace metal content and distributions of certain biological populations; and 3) a zone of active gas seepage near the shelf-slope break. In addition to the transect stations, four stations on each of two submarine carbonate reefs, Hospital Rock and Southern Bank, were sampled in 1976 (Figure 1.4). The collection sites were decreased to two stations at each reef in 1977. Table 1.1 presents the LORAN and LORAC coordinates, latitude, longitude, and depth of each site sampled during the three year study.

Sampling Effort for 1977 Study

Samples were collected in 1977 during three biological-meteorological seasons from all transects and four of the bank stations. The three seasons were winter (January and February), spring (May and June) and fall (September and October). In addition to the seasonal samplings some of the elements sampled Transect II during the six months (March, April, July, August, November and December) not included in the three seasonal sampling periods.

The sampling effort was broken up into four types of sampling: water column, benthos, histopathology and microbiology. Table 1.2 provides a complete list of cruises by date and type of sampling performed. Tables 1.3 - 1.6 give a breakdown of the different scientific elements by sampling frequency . Table 1.7 lists the sampling gear deployed for each sample type. Complete descriptions of sampling methods are included in each work element report.

Navigation and station location for water column, histopathology, and microbiology cruises were by LORAN-A. Navigation and station location

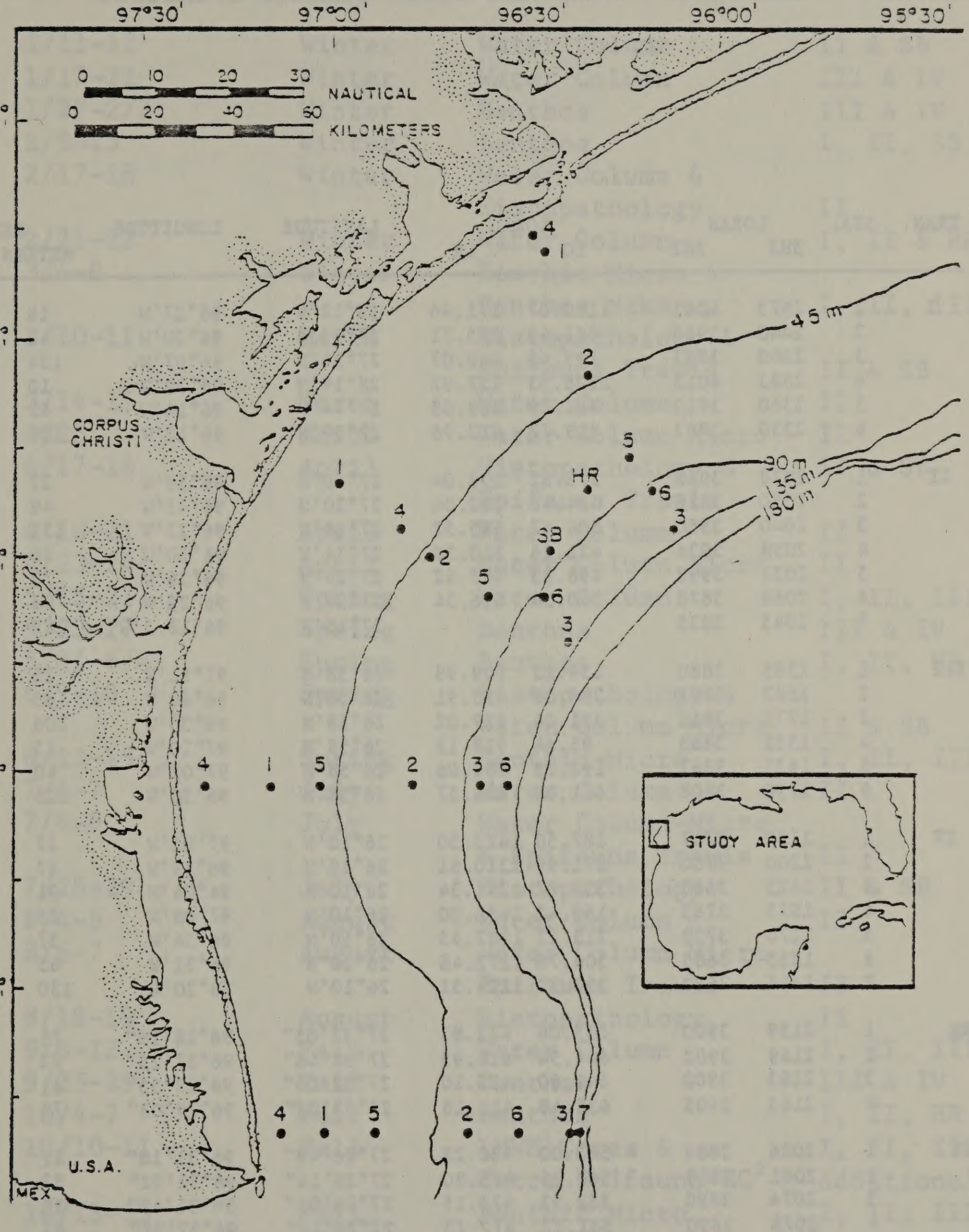

Figure 1.4 Station Locations for the South Texas Outer Continental Shelf Study. Depths in Meters.

TABLE 1.1

BLM STOCS MONITORING STUDY STATION LOCATIONS

TRAN.	STA.	LORAN		LORAC		LATITUDE	LONGITUDE	DEPTH	
		3H3	3H2	LG	LR			METERS	FEET
I	1	2575	4003	1180.07	171.46	28°12'N	96°27'W	18	59
	2	2440	3950	961.49	275.71	27°55'N	96°20'W	42	138
	3	2300	3863	799.45	466.07	27°34'N	96°07'W	134	439
	4	2583	4015	1206.53	157.92	28°14'N	96°29'W	10	33
	5	2360	3910	861.09	369.08	27°44'N	96°14'W	82	269
	6	2330	3892	819.72	412.96	27°39'N	96°12'W	100	328
II	1	2078	3962	373.62	192.04	27°40'N	96°59'W	22	72
	2	2050	3918	454.46	382.00	27°30'N	96°45'W	49	161
	3	2040	3850	564.67	585.52	27°18'N	96°23'W	131	430
	4	2058	3936	431.26	310.30	27°34'N	96°50'W	36	112
	5	2032	3992	498.85	487.62	27°24'N	96°36'W	78	256
	6	2068	3878	560.54	506.34	27°24'N	96°29'W	98	322
	7	2045	3835			27°15'N	96°18.5'W	182	600
III	1	1585	3880	139.13	909.98	26°58'N	97°11'W	25	82
	2	1683	3841	286.38	855.91	26°58'N	96°48'W	65	213
	3	1775	3812	391.06	829.02	26°58'N	96°33'W	106	348
	4	1552	3885	95.64	928.13	26°58'N	97°20'W	15	49
	5	1623	3867	192.19	888.06	26°58'N	97°02'W	40	131
	6	1790	3808	411.48	824.57	26°58'N	96°30'W	125	410
IV	1	1130	3747	187.50	1423.50	26°10'N	97°01'W	27	88
	2	1300	3700	271.99	1310.61	26°10'N	96°39'W	47	154
	3	1425	3663	333.77	1241.34	26°10'N	96°24'W	91	298
	4	1073	3763	163.42	1456.90	26°10'N	97°08'W	15	49
	5	1170	3738	213.13	1387.45	26°10'N	96°54'W	37	121
	6	1355	3685	304.76	1272.48	26°10'N	96°31'W	65	213
	7	1448	3659	350.37	1224.51	26°10'N	96°20'W	130	426
HR	1	2159	3900	635.06	422.83	27°32'05"	96°28'19"	75	246
	2	2169	3902	644.54	416.95	27°32'46"	96°27'25"	72	237
	3	2163	3900	641.60	425.10	27°32'05"	96°27'35"	81	266
	4	2165	3905	638.40	411.18	27°33'02"	96°29'03"	76	250
SB	1	2086	3889	563.00	468.28	27°26'49"	96°31'18"	81	266
	2	2081	3889	560.95	475.80	27°26'14"	96°31'02"	82	269
	3	2074	3890	552.92	475.15	27°26'06"	96°31'47"	82	269
	4	2078	3890	551.12	472.73	27°26'14"	96°32'07"	82	269

TABLE 1.2

SCHEDULE OF CRUISES

Cruise Number	Dates	Sampling Period	Cruise Type	Transects
51	1/11-12	Winter	Water Column	II & SB
53	1/17-22	Winter	Water Column	III & IV
54	1/27-2/3	Winter	Benthos	III & IV
55	2/9-15	Winter	Benthos	I, II, SB & HR
56	2/17-18	Winter	Water Column & Histopathology	II
57	2/21-22	Winter	Water Column	I, II & HR
60	3/4-8	Winter	Benthic Micro & Benthos Make-up	I, II, III & IV
61	3/10-11	March	Histopathology & Epifauna Trawls	II & SB
62	3/14-15	March	Water Column	II
63	3/25	March	Water Column Micro	II
64	4/17-18	April	Histopathology & Epifauna Trawls	II & SB
65	4/20-21	April	Water Column	II
66	4/23-24	April	Water Column Micro	II
67	5/15-21	Spring	Water Column	I, II, III & IV
68	5/23-27	Spring	Benthos	III & IV
69	5/21-6/4	Spring	Benthos	I, II, HR & SB
70	6/9-10	Spring	Histopathology & Water Column Micro	II & SB
71	6/13-15	Spring	Benthic-Micro	I, II, III & IV
72	7/6-7	July	Water Column	II
73	7/8-9	July	Water Column-Micro & Epifauna Trawls	II
74	7/28-29	July	Histopathology	II & SB
75	8/4-5	August	Water Column	II
76	8/6-7	August	Water Column Micro Epifauna Trawls	II
77	8/18-19	August	Histopathology	II
78	9/6-12	Fall	Water Column	I, II, III & IV
79	9/25-29	Fall	Benthos	III & IV
80	10/4-7	Fall	Benthos	I, II, HR & SB
81	10/10-11	Fall	LMWH[1] Cores & Extra Epifauna HC[2]	I, II, III, IV & additional stations
82	10/15-18	Fall	Benthic Micro	I, II, III, IV
83	10/20-21	Fall	Histopathology & Water Column Micro	II
84	11/3-4	Fall	LMWH Cores	I, II, III, IV & additional stations
85	11/5-6	November	Water Column	II
86	11/18-19	November	Histopathology	II & SB
87	11/20-21	November	Water Column & Epifauna Trawls	II

TABLE 1.2 CONT.'D

Cruise Number	Dates	Sampling Period	Cruise Type	Transect
88	11/20-12/1	November	Epifauna Trawls Make-up	II
89	12/2-3	December	Water Column	II
90	12/14-15	December	Histopathology	II & SB
91	12/16	December	Water Column Micro & Epifauna Trawls	II
92	12/18-19	December	Epifauna Trawls Make-up	II

SB - Southern Bank
HR - Hospital Rock
[1] Low-Molecular-Weight Hydrocarbons
[2] Epifauna hydrocarbons

TABLE 1.3
WATER COLUMN SAMPLING

Sample Type	Collected Seasonally[1]	Stations	Collected Monthly[2]	Stations	Collected Periodically	Stations
Meteorology	X	All stations occupied	X	All stations occupied	X	All stations occupied
STD Profiles	X	All transect stations & two bank stations	X	Stations 1-6, Transect II		
Light Penetration	X	Stations 1-3, Transects I, III & IV: Stations 1-6, Transect II; & two bank stations	X	Stations 1-6, Transect II		
Dissolved Oxygen	X	Stations 1-3; Transects I, III & IV; Stations 1-6, Transect II; & two bank stations	X	Stations 1-6, Transect II		
Nutrients	X	Stations 1-3, all transects, & two bank stations	X	Stations 1-3, Transect II		
Dissolved LMW[3] Hydrocarbons	X	Stations 1-3, all transects	X	Stations 1-3, Transect II		
Dissolved and Particulate HMW[4] Hydrocarbons	X	Stations 1-3, all transects				
Particulate Trace Metals	Winter & Spring	Stations 1 and 3, all transects				
Chlorophyll *a*	X	Stations 1-3, all transects	X	Stations 1-3, Transect II		
Fluorescence Transects	X	Along Transect II	X	Along Transect II		
Phytoplankton						
a. C^{14} Productivity	X	Stations 1-3, all transects	X	Stations 1-3, Transect II		
b. Taxonomy	X	Stations 1-3, all transects	X	Stations 1-3, Transect II		
Shelled Microzooplankton	X	Stations 1-3, all transects	X	Stations 1-3, Transect II		
Ciliated Protozoa	X	Stations 1-3, all transects	X	Stations 1-3, Transect II		
Zooplankton						
a. Taxonomy	X	Stations 1-3, all transects	X	Stations 1-3, Transect II		
b. Trace Metals	X	Stations 1-3, all transects	X	Stations 1-3, Transect II		
c. Hydrocarbons	X	Stations 1-3, all transects	X	Stations 1-3, Transect II		
Neuston	X	Stations 1-3, all transects	X	Stations 1-3, Transect II		
Currents					X	Two studies during the year (see Chapter 2)

[1] Winter (Jan-Feb), Spring (May-June), Fall (Sept-Oct)
[2] March, April, July, August, November, December
[3] Low-Molecular-Weight
[4] High-Molecular-Weight

TABLE 1.4

BENTHOS SAMPLING

Sample Type	Collected Seasonally[1]	Stations	Collected Monthly[2]	Stations	Collected Periodically	Stations
Meteorology	X	All stations occupied	X	All stations occupied	X	All stations occupied
Sediment-Texture	X	All transect and bank stations	X	Stations 1-6, Transect II	X	From sediment HMWH samples
Sediment Total Organic Carbon	X	All transect and bank stations				
Sediment Delta C^{13}	X	All transect and bank stations				
Sediment HMW[3] Hydrocarbons					X	Stations 1-3, all transects (each station sampled once during the year)
Sediment LMW[4] Hydrocarbons					X	50 stations in STOCS area (each station sampled once during the year)
Sediment Trace Metals	X	All transect stations				
Meiofauna	X	All transect and bank stations	X	Stations 1-6, Transect II		
Macroinfauna	X	All transect and bank stations				
Macroepifauna and Demersal Fish						
a. Taxonomy	X	All transect stations	X	Stations 1-3, Transect II		
b. Trace Metals	X	Stations 1-3, all transects			X	Stations 1-3, all transects
c. Hydrocarbons	X	Stations 1-3, all transects				
Macronekton						
a. Trace Metals	X	Hospital Rock and Southern Bank				
b. Hydrocarbons	X	Hospital Rock and Southern Bank	X	Southern Bank		

[1] Winter (Jan-Feb), Spring (May-June), Fall (Sept-Oct)
[2] March, April, July, August, November, December
[3] Low-Molecular-Weight
[4] High-Molecular-Weight

TABLE 1.5

HISTOPATHOLOGY SAMPLING

Sample Type	Collected Seasonally[1]	Stations	Collected Monthly[2]	Stations
Meteorology	X	All stations occupied	X	All stations occupied
Histopathology a. Macroepifauna	X	Stations 1-3, Transect II	X	Stations 1-3, Transect II
b. Demersal Fish	X	Stations 1-3, Transect II and Southern Bank	X	Stations 1-3, Transect II and Southern Bank
c. Gonadal Tissues	X	Stations 1-3, Transect II and Southern Bank	X	Stations 1-3, Transect II and Southern Bank

[1] Winter (Jan-Feb), Spring (May-June), Fall (Sept-Oct)
[2] March, April, July, August, November, December

TABLE 1.6

MICROBIOLOGY SAMPLING

Sample Type	Collected Seasonally[1]	Stations	Collected Monthly[2]	Stations
Meteorology	X	All stations occupied	X	All stations occupied
Mycology a. Water Column			X	Stations 1-3, Transect II
b. Benthic	X	Stations 3/I, 1/II, 2/II 3/II, 2/III and 1/IV		
Bacteriology a. Water Column	X	Stations 1-3, Transect II	X	Stations 1-3, Transect II
b. Benthic	X	Stations 1-3, all transects		
Sediment Texture	X	Stations 1-3, all transects		

[1] Winter (Jan-Feb), Spring (May-June), Fall (Sept-Oct)

[2] March, April, July, August, November, December

TABLE 1.7

SAMPLING GEAR USED DURING THE 1977 STOCS STUDY

Type Of Sampling	Gear Used
STD Profiles	Plessey Salinity/Temperature/Depth Profiling System and Nansen Bottles Equipped with Reversing Thermometers
Light Penetration	Lambda Photometer or Secchi Disc
Dissolved Oxygen	Nansen Bottles Equipped with Reversing Thermometers
Nutrients	30-ℓ Niskin Bottles
Dissolved LMW^1 Hydrocarbons	30-ℓ Niskin Bottles
Dissolved and Particulate HMW^2 Hydrocarbons	19-ℓ Glass Carboy in Stainless Steel Cage
Particulate-Trace Metals	19-ℓ Plastic Carboy
Chlorophyll *a*	30-ℓ Niskin Bottles
Fluorescence Transects	
Phytoplankton	
a. C^{14} Productivity	30-ℓ Niskin Bottles
b. Taxonomy	30-ℓ Niskin Bottles
Shelled Microzooplankton	
a. Discrete Depth	30-ℓ Niskin Bottles
b. Integrated Depth	30-cm Nansen Net, 76 μm Mesh
Ciliated Protozoa	30-ℓ Niskin Bottles
Zooplankton	
a. Taxonomy	1-m dia., 250 μm Mesh Net, equipped with a Flowmeter and a Time-Depth Recorder
b. Trace Metals and HMW Hydrocarbons	1-m dia., 250 μm Mesh Net, with PVC Frame, Towed with a Nylon Rope from a Boom at the Side of Survey Vessel
Neuston	2 x .5 m Frame Partitioned into Four Equal Areas with Four 505 μm Mesh Nets
Sediment Texture	Smith-McIntyre Grab Sampler (.0125 m^3)
Sediment Total Organic Carbon	Smith-McIntyre Grab Sampler (.0125 m^3)
Sediment Delta C^{13}	Smith-McIntyre Grab Sampler (.0125 m^3)

TABLE 1.7 CONT.'D

Type of Sampling	Gear Used
Sediment HMW Hydrocarbons	Smith-McIntyre Grab Sampler (.0125 m^3)
Sediment Trace Metals	Smith-McIntyre Grab Sampler (.0125 m^3)
Meiofauna	Smith-McIntyre Grab Sampler (.0125 m^3)
Macroinfauna	Smith-McIntyre Grab Sampler (.0125 m^3)
Macroepifauna Demersal Fish	
a. Taxonomy	35-ft (10.7 m) Otter Trawl
b. Trace Metals	35-ft (10.7 m) Otter Trawl
c. Hydrocarbons	35-ft (10.7 m) Otter Trawl
d. Histopathology	35-ft (10.7 m) Otter Trawl
Macronekton	
a. Trace Metals	Hook and Line
b. Hydrocarbons	Hook and Line
Benthic Mycology	Smith-McIntyre Grab Sampler (.0125 m^3)
Benthic Bacteriology	Smith-McIntyre Grab Sampler (.0125 m^3)
Water Column Mycology	1-ℓ Niskin Sterile Sampler or Peristaltic Pump and Tygon Tubing
Water Column Bacteriology	1-ℓ Niskin Sterile Sampler or Peristaltic Pump and Tygon Tubing
Sediment LMW Hydrocarbons	Gravity Core with Plastic Liner
Current Measurements	ENDECO Type 105 Recording Current Meter

[1] Low-Molecular-Weight
[2] High-Molecular-Weight

for the benthos cruises were by LORAC navigational systems.

A summary of high-molecular-weight hydrocarbons and trace metal quality control samples collected during the 1977 contracting period is given in Table 1.8. Hydrocarbon quality control samples were delivered to the University of New Orleans. Trace metal quality control samples are in storage at UTMSI/PAML, pending the naming of a trace metal quality control laboratory.

Survey Vessel

All sampling and measurements, except the placement and recovery of current meters were taken aboard the University of Texas research vessel, the R/V LONGHORN. The R/V LONGHORN, designed and constructed as a coastal research vessel in 1971, is a steel-hulled 24.38 (80 ft) by 7.42 m (24 ft), 2.13 m (7 ft) draft ship. She carries a crew of five and can accomodate a scientific party of ten. The R/V LONGHORN is equipped with a stern-mounted crane, a trawling winch, scan sonar, radar, LORAN-A and LORAC navigational systems, and dry and wet laboratory space.

Participants

The University of Texas Marine Science Institute, Port Aransas Marine Laboratory (UTMSI/PAML), was contracted by the BLM to provide overall project management, logistics, ship time, data management and certain scientific efforts. Additional scientific effort was provided by sub-contracts between the University of Texas and Texas A&M University, The University of Texas at San Antonio, The University of Texas at Austin, and Rice University.

A total of 28 principal investigators participated in the project. Table 1.9 lists the principal investigators by institutions represented and scientific responsibility. Ship time was provided for the NOAA/NMFS

TABLE 1.8

SUMMARY OF QUALITY CONTROL SAMPLES COLLECTED UNDER BLM CONTRACT AA550-CT -1

Sample Type	Hydrocarbon		Trace Metal	
	Contracted	Collected	Contracted	Collected
Macroepifauna and Demersal Fishes	24	24	24	24
Particulate High-Molecular Weight Hydrocarbons	12	12	0	0
Zooplankton	12	12	12	12
Macronekton	4	4	4	4
Sediment	24	24	24	24
Ship's Contaminants	9	9	3	3

TABLE 1.9

STOCS BIOLOGICAL AND CHEMICAL COMPONENT PARTICIPANTS BY WORK ELEMENT AND INSTITUTION

Rice University	
Microplankton and Shelled Microzoobenthon	R. E. Casey
Texas A&M University	
HMW Hydrocarbons in Macroepifauna, Demersal Fish and Macronekton	C. S. Giam, H. S. Chan
Trace Metals in Macroepifauna, Demersal Fish Macronekton and Plankton	B. J. Presley, P. N. Boothe
LMW Hydrocarbons, Nutrients and Dissolved Oxygen	W. M. Sackett, J. M. Brooks
Zooplankton	E. T. Park
Neuston	J. H. Wormuth
Meiofauna	W. E. Pequegnat
Histopathology of Macroepifauna	J. M. Neff
Histopathology of Demersal Fishes	W. E. Haensly
Benthic Bacteriology	J. R. Schwarz
University of Texas	
Austin:	
Water Column and Benthic Mycology	P. J. Szaniszlo
Marine Science Institute / Galveston Geophysical Laboratory:	
Sediment Texture	E. W. Behrens
Marine Science Institute/Port Aransas Marine Laboratory:	
Ciliated Protozoa	P. L. Johansen
Hydrography	N. P. Smith
HMW Hydrocarbons in Zooplankton, Sediment, Water	P. L. Parker, R. S. Scalan, J. K. Winters
Phytoplankton and Productivity	C. Van Baalen, D. L. Kamykowski
Macroinfauna and Macroepifauna	J. S. Holland
Demersal Fishes	D. E. Wohlschlag
San Antonio:	
Histopathology: Gonadal Tissues of Macroepifauna and Demersal Fish	S. A. Ramirez
Water Column Bacteriology	M. N. Guentzel, H. V. Oujesky, O. W. Van Auken

ichthyoplankton sampling. Supportive work was performed by the USGS (sediment texture and sediment trace metals) and the Topographic Features (sediment texture) components.

Program Management

For the third year of environmental studies of the STOCS, the biological and chemical component required a full-time Program Manager and staff. The Program Management staff consisted of a Technical Coordinator, Data Manager, Program Secretary, marine technician, draftsperson and ancillary data management personnel. The primary responsibilities of the Program Manager and staff included overall program administration, logistical coordination for field sampling, sample transmittals, lab analyses, data management and meetings, and preparation of required reports.

Meetings were held at the end of each quarter of the contracting period. All Principal Investigators for the biological and chemical component and element leaders of other STOCS projects presented a summary of significant findings and progress reports at these meetings. Following each quarterly conference a Quarterly Summary Report was submitted to BLM. These reports summarized the following: all work accomplished and problems encountered; an updated sample inventory showing all samples taken and their disposition; and the Principal Investigators' quarterly progress reports. To insure communication, coordination and unity of effort, an Administrative Council [consisting of the Program Manager, Technical Coordinator, Data Manager, the Project Element Coordinators of other STOCS projects and the Contracting Officer's Authorized Representative (COAR)] met prior to each quarterly conference.

Problems

The usual weather and mechanical problems associated with offshore sampling were encountered and reported to BLM in cruise reports submitted following each cruise. All field sampling was, however, completed in less time than originally estimated. Specific field sampling problems, as well as laboratory analytical and taxonomic problems, are discussed in detail in each of the reports by the Principal Investigators.

Publications/Works in Progress

A number of publications and presentations have resulted from the 1975-1977 STOCS studies. These, along with works in progress, are presented in Table 1.10. It is anticipated that the number and rate of publications will increase as additional data are available and analyzed.

TABLE 1.10

PUBLICATIONS AND PRESENTATIONS

HYDROGRAPHY

Smith, N. P. 1978. Longshore currents on the fringes of Hurricane Anita. J. Geophys. Res. 1977 Data.

________. 1977. Nearshore cross-shelf motion in the northwestern Gulf of Mexico. Presented to the Am. Geophys. Un. June 1977, Washington, D. C.

________. 1977. Longshore coherence in nearshore motion. Presented at the Am. Geophys. Un. Fall, 1977.

________. 1978. Vertical coherence in wind-driven shelf motions. (Based on spring circulation study). Submitted to the J. of Phys. Res.

________. In Preparation. Cross-shelf variability in nearshore circulation. (Based on rig monitoring current and wind data).

________. In Preparation. Longshore coherence in nearshore motion. (Based on 1976 early summer circulation study).

________. In Preparation. Cross-shelf variability in shelf circulation. (Based on 1976 late summer circulation study).

LOW-MOLECULAR-WEIGHT HYDROCARBONS, NUTRIENTS AND DISSOLVED OXYGEN

Brooks, J. M., D. F. Reid, B. B. Bernard. 1977. Methane association with shallow nepheloid layers in the Northwest Gulf of Mexico. Presented at the AGU Spring Meeting, San Francisco, December 5-9, 1977.

Brooks, J. M. *et al.* 1977 (In Press). Light hydrocarbons in recent Texas continental shelf and slope sediments. J. Geophysical Res.

Brooks, J. M., B. B. Bernard, and W. M. Sackett. 1976. Input of low-molecular-weight hydrocarbons from petroleum operations into the Gulf of Mexico. Presented at the Fates and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organism Meeting. 10-12 November 1976. Seattle, Washington, D. C.

HIGH-MOLECULAR-WEIGHT HYDROCARBONS IN SEDIMENTS, ZOOPLANKTON AND WATER

Fry, B., R. S. Scalan, and P. L. Parker. 1977. Stable carbon isotope evidence for two sources of organic matter in coastal sediments, sea grasses and plankton. Geochim. et Cosmochimica Acta 41:1875-1877.

TABLE 1.10 CONT.'D

Parker, P. L. 1976. Petroleum hydrocarbons in Gulf of Mexico coastal waters: chemical characteristics and biological effects. Presented at the AIBS Symposium on Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment. Washington, D. C. August 9-11, 1976.

________. 1976. Preliminary results of a chemical and biological survey of the south Texas shelf. Abstr. Prog. Geol. Soc. Am. 8:57.

________., J. K. Winters, C. Van Baalen, J. C. Batterton, and R. S. Scalan. 1976. Petroleum Pollution: Chemical Characteristics and Biological Effects. Presented at the AIBS Symposium on Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment. Washington, D. C. August 9-11, 1976.

Scalan, R. S. 1976. Hydrocarbons in zooplankton from the STOCS. Presented to the Southern Regional Geochemists. Tulsa, Oklahoma. May 14, 1976.

Winters, J. K., and P. L. Parker. 1977. Water soluble components of crude oils, fuel oils and used crankcase oils. Presented at the Oil Spill Conference. New Orleans, Louisiana March 8-10, 1977.

HIGH-MOLECULAR-WEIGHT HYDROCARBONS IN EPIFAUNA AND MACRONEKTON

Giam, C. S., H. S. Chan, and G. S. Neff. 1976. Aliphatic heavy hydrocarbon composition in the benthic macroepifauna of the South Texas outer continental shelf. Presented at the 32nd annual ACS Southwestern Meeting. Fort Worth, Texas December 1-3, 1977.

___________., 1976. Distribution of n-paraffins in selected marine benthic organisms. Bull. Env. Contam. & Toxicol. Vol. 16(1): 37-43. Springer & Verlag, New York.

TRACE METAL CONCENTRATIONS IN EPIFAUNA, ZOOPLANKTON AND MACRONEKTON

Horowitz, A., and B. J. Presley. 1977. Trace metal concentrations and partitioning in zooplankton, neuston and benthos from the STOCS. Arch. of Env. Contam. & Toxicol. 5(2):247-255.

Sims, R. R., and B. J. Presley. 1976. Heavy metal concentrations in organisms from an actively dredged Texas bay. Bull. Env. Contam. & Toxicol. 16(5):520-527.

SEDIMENT TEXTURE

Behrens, E. W. 1978. (In Press) Further comparisons of grain size distribution determined by electronic particle counting and pipette techniques. J. Sedimentary Petrology

TABLE 1.10 CONT.'D

MICROBIOLOGY: BENTHIC BACTERIOLOGY, WATER COLUMN BACTERIOLORY, AND BENTHIC AND WATER COLUMN MYCOLOGY

Alexander, S. K., H. S. Chan, C. S. Giam, and J. R. Schwarz. 1978. Effect of crude oil on benthic bacteria of the South Texas outer continental shelf. Presented at the Annual Meeting of the American Society for Microbiology, Las Vegas, Nevada. May 14-19, 1978.

Kaster, A. G., J. Allen, and O. W. Van Auken. 1978. Species trends in bacterial succession in water samples from the western Gulf of Mexico. Presented at the Texas Academy of Science Meeting, Lubbock, Texas, March 9, 1978.

_________., and O. W. Van Auken. 1978. Bacterial succession in closed systems incubated with and without South Louisiana crude oil. Presented at the American Society for Microbiology, Las Vegas, Nevada. May 14-19, 1978.

Oujesky, H. V. 1978. Water column bacteriological studies of the south Texas outer continental shelf. To be presented at the Annual Meeting of the Society for Industrial Microbiology in Houston, August 13-18, 1978. To be published in Developments in Industrial Microbiology, Vol. 20, Spring 1979.

Pouw, H., E. Ley, and O. W. Van Auken. 1978. Some effects of South Louisiana crude oil on bacterial kinetics. Presented at the Texas Academy of Science Meeting, Lubbock, Texas, March 9, 1978.

Van Auken, O. W., R. Lutes, and P. L. Parker. 1978. Hydrocarbon degrading potentials of water samples from the western Gulf of Mexico. Presented at the Texas Academy of Science Meeting, Lubbock, Texas, March 9, 1978.

_________., and A. G. Kaster. 1978. Iron requirements of oil-degrading bacteria from western Gulf of Mexico. Presented at the American Society for Microbiology, Las Vegas, Nevada, May 14-19, 1978.

Wilson, C., A. G. Kaster, and O. W. Van Auken. 1978. Some effects of South Louisiana crude oil on bacterial succession. Presented at the Texas Academy of Science, Lubbock, Texas, March 9, 1978.

PHYTOPLANKTON AND PRODUCTIVITY

Morgan, J. C. 1975. Studies on the diatoms of the northwestern Gulf of Mexico: identification, distribution and cultural studies. M.A. Thesis. University of Texas, Austin, Texas. 67pp.

MICROZOOPLANKTON AND MICROZOOBENTHON

Anepohl, J. K. 1976. Seasonal occurrence of living benthonic Foraminifera, south Texas outer continental shelf. Abstr. Prog.

TABLE 1.10 CONT.'D

Geol. Soc. Am. 8:2-3 (Abstr).

Anepohl, J. K. 1976. Seasonal distribution of living benthonic Foraminifera of the south Texas outer continental shelf. M. A. Thesis, Univ. of Texas, Austin, Texas. 130pp.

Bauer, M. A. 1976. Ecology and distribution of living planktonic foraminifera of the south Texas outer continental shelf. M. A. Thesis, Rice University., Houston, Texas 125pp.

Casey, R. E. 1977. Pre-drilling environmental impact studies on the south Texas outer continental shelf. Presented at the Corp. Association Meeting, Houston, December 1977.

________. 1977. Cenozoic radiolarians: paleogeography and evolution Presented at the N. Am. Paleontol. Convention., II, University of Kansas, August 1977.

________., and M. A. Bauer. 1976. A seasonal study of radiolaria and foraminifera in the waters overlying the south Texas outer continental shelf. Abstr. Prog. Geol. Soc. Am. 8:11 (Abstr).

________., and K. J. McMillen. In Press. Cenozoic radiolarians of the Atlantic basin margins. *In* F. Swain (ed)., Stratographic micropaleontology of the Atlantic Basin and borderlands.

________., K. J. McMillen, and M. A. Bauer. 1975. Evidence for and paleo-oceanographic significance of relict radiolarian populations in the Gulf of Mexico and Caribbean. Abstr. Prog. Geol. Soc. Am. 7:1012-1023 (Abstr).

Hueni, C., J. A. Monroe, J. Gevirtz, and R. E. Casey. 1978. Distribution of living benthonic foraminifera as indicators of oceanographic processes of the south Texas outer continental shelf. To be presented at the Gulf Coast Association of Geological Societies, New Orleans, Louisiana Oct. 11-13, 1978.

________., and R. E. Casey. 1977. Benthonic foraminifera from the south Texas outer continental shelf; a seasonal study for 1976. Presented at the Offshore Technology Conference, Houston,-Texas May 1978.

Leavesley, A. S., M. A. Bauer, K. McMillen, and R. E. Casey. 1978. Living shelled microzooplankton (radiolarians, foraminiferans, and pteropods) as indication of oceanographic processes in water over the outer continental shelf of south Texas. To be presented at the Gulf Coast Association of Geological Societies New Orleans, Louisiana Oct. 11-13, 1978.

________., and R. E. Casey. 1977. Shelled microzooplankton as indicators of oceanographic conditions on the south Texas outer continental shelf, 1976. Presented at the Offshore Technology Conference, Houston, Texas May 1978.

TABLE 1.10 CONT.'D

CILIATED PROTOZOA

Johansen, P. L. 1978. Factors affecting the distribution of marine protozoa on the south Texas outer continental shelf. Presented at the ASLO Meeting, Victoria, British Columbia, June 19-22, 1978.

________. 1977. Microdistribution in time and space of tintinnids and other marine protozoa. Presented at the V International Congress of Protozoology, New York., June 26-July 2, 1977.

NEUSTON

Pequegnat, L. H. 1979. Pelagic tar concentrations in the Gulf of Mexico over the south Texas outer continental shelf. Submitted to the Contr. in Mar. Sci. Vol 22.

Wormuth, J. H. 1978. Sampling variability associated with the neuston community: the results of an intensive two-year study on the south Texas outer continental shelf. Presented at the Scientific Committee of Ocean Resources and the Scientific Committee in Antarctic Resources. Keil, Germany, May 30-June 2, 1978.

________., and S. P. Berkowitz. 1977. Annual changes in the neuston of the south Texas outer continental shelf and their relationship to other physical and biological variables. Presented at the annual ASLO meetings, East Lansing, Michigan, June 20-23, 1977.

INVERTEBRATE EPIFAUNA AND MACROINFAUNA

Flint, R. W., and J. S. Holland. 1978. Benthic species variability in a marine nearshore subtidal environment. Presented at the ASLO Meeting, Victoria, British Columbia, June 19-22, 1978, Paper submitted to Marine Biology for publication.

________., S. A. Holt, and J. S. Holland. In Preparation. Gulf of Mexico outer continental shelf benthos: II Macroinfaunal environmental relationships.

Holland, J. S., and S. A. Holt. In Preparation. Estimating accuracy and efficiency of marine benthic sampling.

Holt, G., and J. S. Holland. In Preparation. Epifaunal community structure of the south Texas outer continental shelf.

Holt, S. A., R. W. Flint, and J. S. Holland. In Preparation. Gulf of Mexico outer continental shelf benthos: I. Macroinfaunal species assemblages and distribution.

McKinney, L., R. D. Kalke, and J. S. Holland. 1978. New species of amphipods from the western Gulf of Mexico. Contr. Mar. Sci. 21: 133-159.

TABLE 1.10 CONT.'D

Rabalais, N., and S. A. Holt. 1978. The mud shrimps (Crustacea, Decapoda, Thalassinidea) of the northwestern Gulf of Mexico. Presented at the Texas Academy of Science Meeting, Lubbock, Texas, March 9, 1978

________., In Manuscript. A new species of Ctenocheles (Crustacea: Decapoda, Thalassinidea) from the northwestern Gulf of Mexico. Submitted to Proc. Biol. Soc., Washington.

________., S. A. Holt, and J. S. Holland. In manuscript. The Thalassinids (Crustacea, Decapoda) from the northwestern Gulf of Mexico. To be submitted to Bull. Mar. Sci.

________., and D. L. Felder. In Preparation. The Brachyuran crabs (Crustacea, Decapoda) of the south Texas continental shelf.

________________. In Preparation. The Anomuran crabs (Crustacea, Decapoda) of the south Texas continental shelf.

________________. In Preparation. The shrimps (Crustacea, Decapoda, Natantia) of the south Texas continental shelf.

________________. In Preparation. A quantitative, diversity, population paper on all decapods.

Treece, G., and J. S. Holland. In Press. Four new records of Aplacophorous mollusks from the Gulf of Mexico. Bull. of Mar. Sci. 14 pp. 19pl.

DEMERSAL FISHES

Cole, J. F. 1977. Numerical analysis of trawled benthic fishes from the south Texas outer continental shelf. M. A. Thesis. Univ. of Texas, Austin, Texas.

Vetter, E. F. 1978. Diel variations in otter trawl vulnerability of fishes from the south Texas outer continental shelf. M. A. Thesis, University of Texas, Austin, Texas.

________. 1977. A functional model of *Stenotomus caprinus* (Pisces: Sparidae) distribution and abundance on the south Texas outer continental shelf. Presented at the AIBS Conference, on Assessment of Ecological Impacts of Oil Spills, Keystone, Colorado. June 14-17, 1977.

Wohlschlag, D. E. 1977. Seasonal and annual contrasts in distribution and abundance indices for south Texas continental shelf fishes. Presented at the Annual AIBS Meetings, East Lansing, Michigan, August 1977.

TABLE 1.10 CONT.'D

Wohlschlag, D. E. 1976. Distribution and abundance of STOCS benthic fishes. Presented at the annual ASLO Meeting. Savannah, Georgia., June 21-24, 1976.

________. 1976. Benthic fish distribution and abundance patterns over the south Texas outer continental shelf. Presented at the GERS Meeting., Lafayette, Louisiana. October 30, 1976.

HISTOPATHOLOGY

Ernst, V. V., and J. M. Neff. 1978. Gill histopathologies of various Gulf crustaceans. Presented at the Crustacean Health Workshop, Galveston, Texas April 20-22, 1978. To be published in the proceedings, D. W. Lewis (ed.).

Ramirez, S. A. 1978. Comparative karyotypes of two species of blue crabs, *Callinectes* sp. Presented at the Genetics Society of America, Univ. of South Carolina, June 4-7, 1978.

________. 1977. Histological and cytochemical study of ovarian follicle cells of the squid, *Loligo pealei*. Presented at the American Society for Cell Biologists, San Diego, Calif. November 15-18, 1977.

DATA MANAGEMENT

Whitehead, J. M., J. Holt and E. F. Vetter. In Preparation. Diffusion versus migration: Mathematical-physics methods applied to behaviour problems in biology. Amer. J. of Physics.

________., E. F. Vetter, and J. Holt. In Preparation. Fermi statistics: general applicability in ecological studies. Biometrics.

LITERATURE CITED

Galtsoff, P. S. 1954. Gulf of Mexico, Its origin, waters and marine life. Fishery Bull. 55. U.S. Fish and Wildl Ser.

Gunter, G. 1945. Marine fishes of Texas. Publ. Inst. Mar. Sci. 1:1-190.

Hedgepeth, J. W. 1953. An introduction to the zoogeography of the northwestern Gulf of Mexico with reference to the invertebrate fauna. Publ. Inst. Mar. Sci. 2:7-124.

CHAPTER TWO

HYDROGRAPHIC PROJECT

University of Texas Marine Science Institute
Port Aransas Marine Laboratory

Principal Investigator:

Ned P. Smith

Associate Investigators:

James Evans
William MacNaughton

ABSTRACT

Recording current meter data from two field experiments, and hydrographic data from nine nearly synoptic cruises over the South Texas Outer Continental Shelf were used to describe the annual progression of physical oceanographic variables for 1977, and to infer the dominant time scales and external forcing mechanisms. Current measurements revealed a strong longshore transport to the south-southwest during the winter months and little net transport during the summer. Surface temperature data indicated a transition from strong cross-shelf gradients in the winter to nearly isothermal conditions in summer. Salinity variations were largely restricted to the transient decrease produced by the spring runoff. The principal external forcing mechanisms appeared to be the winter cold fronts, the spring runoff and summer heating.

INTRODUCTION

Shelf waters in general may be characterized by relatively great temporal and spatial variability, reflecting both advective processes (outflow from adjacent estuaries and shelf-open ocean exchanges) and local conditioning through the air-sea interface. The relatively rapid response of shallow shelf waters to external, meteorological forcing greatly restricts the representativeness of a given measurement, both in space and time. As a result, sampling programs must be appropriately designed with regard to station density and sampling frequency to reveal the constantly changing patterns.

The waters of the South Texas Outer Continental Shelf (STOCS) provide a good example of this characteristic of shelf waters. Though the hydrographic climate is quite well known for the central Texas coast and has been reviewed by several authors (Rivas, 1968; Etter and Cochrane, 1975; Armstrong, 1975), the variability or scatter about the long-term averages is much less well understood.

The purpose of the hydrographic component of the STOCS monitoring program was two-fold. The primary purpose was to provide supportive data in a basically chemical and biological investigation. Temperature and salinity measurements provided a data base suitable for going a step beyond simply inventorying plant and animal populations, and made it possible to investigate the cause-and-effect relationships which produced the observed biological distributions.

A second purpose of the study related to the spatial and temporal variability of hydrographic properties in the Texas OCS study area. Repetitive sampling within a relatively closely spaced grid of stations made possible a study of the heterogeneity that was masked by the long-term averages. The importance of investigating temporal and spatial variability was that the physical processes responsible for defining the general hydrographic climate were generally both localized and transient. If the study was to contribute to an understanding of basic processes, the sampling pattern must be appropriately tailored to the characteristics of the physical environment. The 1977 hydrographic sampling program seemed to have been well suited for fulfilling both its intended purposes.

For many purposes, the first year of the monitoring program was the 1976 study, since the poor sampling frequency and station density during the 1975 monitoring did not produce a data base sufficient for more than a first look at large-scale, seasonal patterns. Even with the 1976 hydrographic data, however, there remained the question of year-to-year repetition of hydrographic events or general patterns. With the follow-up data presented in this report, it became possible to draw tentative conclusions regarding the representativeness of a single year's data. The question of year-to-year variability, however, could only be confidently resolved with data from a much longer monitoring study.

The following section contains a description of the methodology used both in data collection and data analysis. These techniques have been presented in prior reports, and are basically unchanged from those used in the 1976 program (Smith, in Groover, 1977). A compilation of the results of hydrographic and direct current measurements follows. This component of the Annual Report concludes with a short integration of the data accumulated during the 1977 monitoring.

MATERIALS AND METHODS

Data Collection

Both hydrographic data and recording current meter data are presented and discussed in this report. Hydrographic data were normally obtained with a Plessey Model 9060 Self-Contained Salinity/Temperature/Depth Profiling System (STD). In very shallow water, or when a brackish water lens through the upper part of the water column lowered the salinity to below the 30-40 parts-per-thousand (ppt) range of the STD, a Martek Model TDC Metering System (TDC) was used instead. The STD provided surface to near-bottom analog traces, which were digitized at 3-m intervals. The TDC was lowered at approximately 3-m intervals.

Top and bottom temperature and salinity samples were collected by Nansen bottles equipped with pairs of reversing thermometers. Salinities were determined by laboratory analysis following the cruise. Top and bottom calibration data were entered into the computer to correct the raw T-S profiles provided by the STD or TDC.

STD temperatures were read to the nearest 0.01°C; salinities were read to the nearest 0.01 ppt. The precision of the STD is nominally within 0.1°C and 0.05 ppt, for temperature and salinity, respectively. When the TDC was used, temperatures were read to the nearest 0.1°C and conductivities were read to the nearest 0.1 mmho/cm. In reality, however, the precisions are more on the order of 0.2°C and 1 to 2 mmho/cm, depending on the full scale being used. The precision of the salinity computed from the TDC data was estimated to be within 0.5 ppt, incorporating the calibration data. TDC depth was read to the nearest 0.1 m, though this generally required averaging on a rolling boat.

The surface and bottom calibration data were used to calibrate both STD and TDC profiles as the first step in a computer program used to compute a group of temperature-, salinity- and pressure-related hydrographic variables. When TDC data were obtained at a given station, the computer program first calculated the salinity with the equations discussed by Bennett (1976). The basic computer program was that developed by and obtained from the Pacific Environmental and Meteorological Laboratories. Our version has undergone significant modifications to (1) incorporate the calibration T-S data; (2) plot vertical profiles of temperature, salinity and sigma-t; and (3) compute the Brunt-Vaisala cut-off frequency of internal wave oscillations. The program computed sigma-t, potential temperature (°C), the specific volume anomaly (the difference between the specific volume of the sample and that of a water parcel with a temperature of 0°C and a salinity of 35 ppt, in cm^3/gm), the dynamic height (the product of the specific volume anomaly and the pressure, in cm^2/sec^2), the potential energy anomaly (in gm cm^2/sec^2) and the sound speed (in m/sec). Tables of these computed hydrographic variables are presented in Appendix A along with the calibrated T-S profiles.

A total of 135 T-S profiles was obtained during the 1977 sampling program. This total included transect data collected on the monthly and seasonal cruises, bank station data collected seasonally, eight profiles from Station 3/II collected as part of an additional exercise, and profiles from stations 1, 2 and 3/II collected in October to provide better temporal resolution between the fall seasonal cruise (conducted during September) and the November monthly cruise.

Recording current meter data were to be obtained during three separate field exercises, designed to coincide roughly with the three seasonal cruises and at the same time provide data from times of winter cooling and convective mixing, spring run-off, and summer stratification. As a circulation study had been carried out in late winter 1977, as part of the

Rig Monitoring Study, and since the Principal Investigator left the project in November, only two of the three planned studies were in fact carried out. These extended from 9 March to 26 April, and from 14 June to 11 September 1977. The time periods were well suited to detect the effects of the spring run-off and summer thermal stratification. In addition, the positioning of the current meters made possible an investigation of cross-shelf coherence during the spring study and longshore coherence during the summer study.

Current speed and direction were recorded either hourly or half-hourly, and all values were time-integrated (averaged) over the sampling period. The current meter used was the ENDECO Type 105, which has an accuracy of approximately 6 cm/sec and a resolution of 2.6 cm/sec, according to the manufacturer; the directional resolution and accuracy are 7°.

For the spring study, current meters were located at two stations, 6 and 33 km off the central Texas coast near Port Aransas (Figure 2.1). Two current meters at the inner station were positioned 2 and 10 m above the bottom in 15 m of water. The four current meters at the outer stations were positioned 5, 12, 19 and 26 m above the bottom in 33 m of water. During the summer study, two current meters 2 and 10 m above the bottom in approximately 20 m of water, were positioned off Port Aransas and Port O'Connor, Texas, and one current meter, 10 m above the bottom in 18 m of water, was positioned off Port Mansfield (see Figure 2.2).

Methods of Analysis

Calibrated T-S profiles and computed hydrographic variables were presented in a number of ways to characterize the hydrographic climate of the STOCS. Calibration T-S data from the seasonal cruises, when all 26 stations were occupied, were plotted on a plan-view base map and contoured

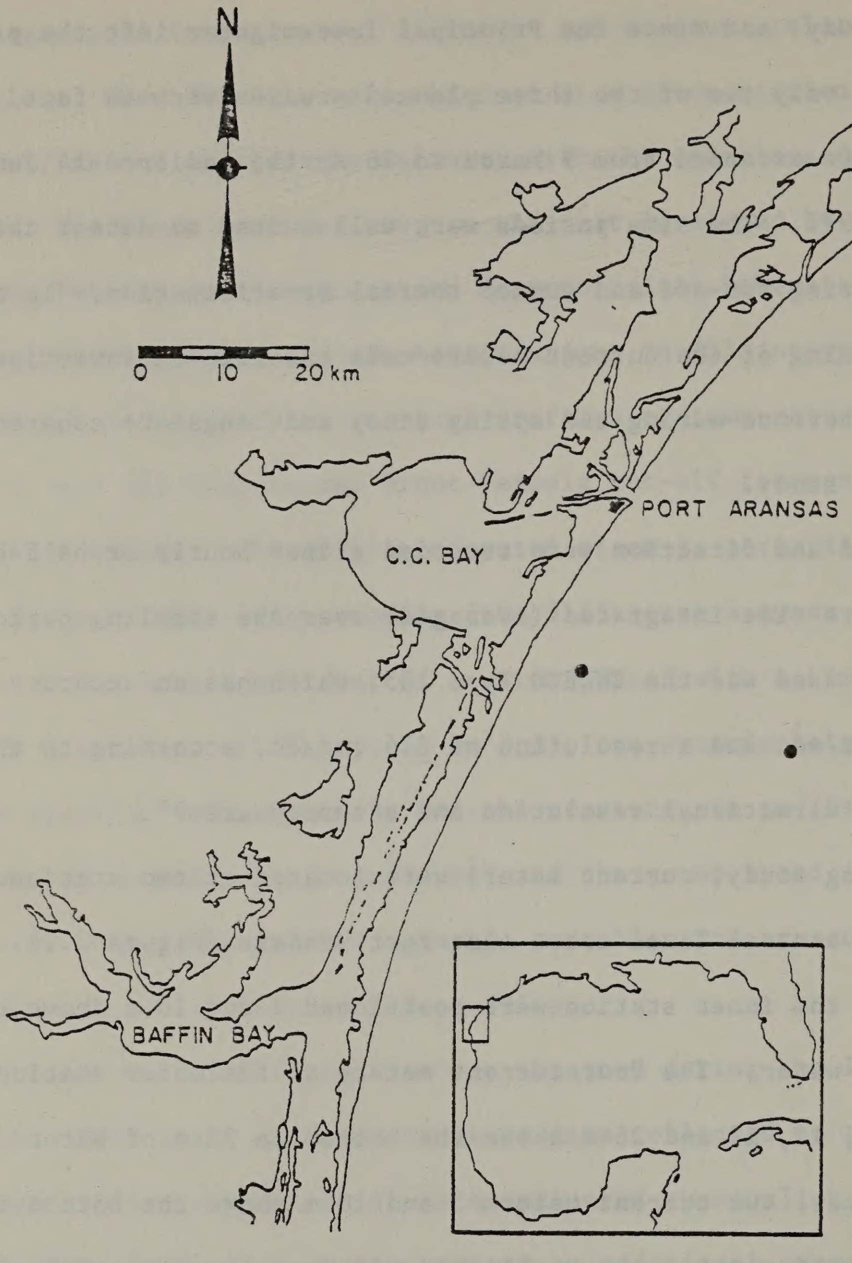

Figure 2.1 Inner and Outer Sampling Sites for Sub-Surface Current Data, March-April 1977.

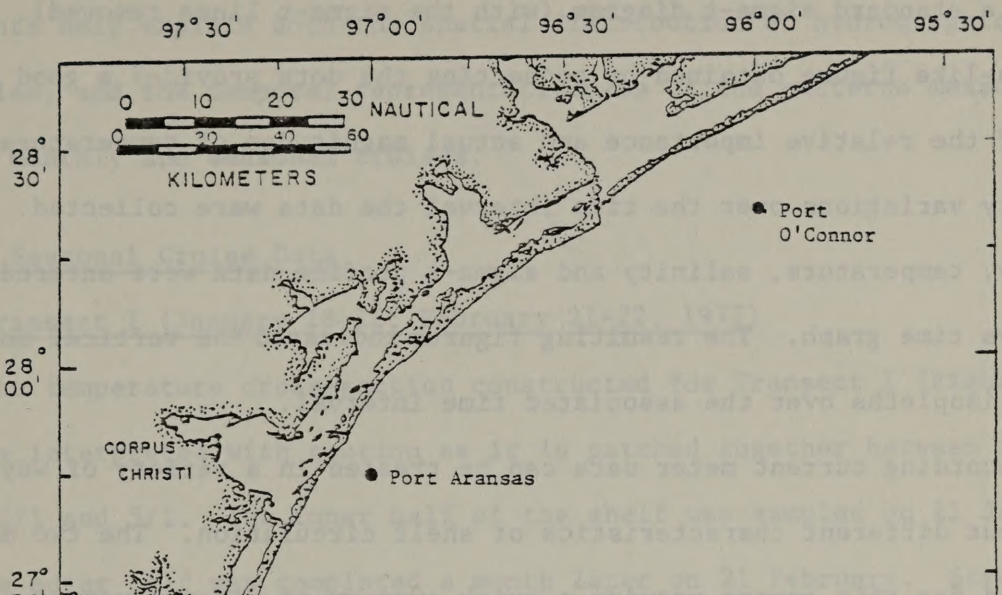

Figure 2.2 Locations of Current Meters off the Texas Gulf Coast.

at intervals of 1-2°C or ppt. Monthly cruise data were used to construct cross-sections of temperature, salinity and sigma-t along Transect II.

T-S values from a given location and depth on Transect II were plotted on a standard sigma-t diagram (with the sigma-t lines removed). The polygon-like figure obtained by connecting the dots provided a good overview of the relative importance and actual magnitudes of temperature and salinity variations over the time interval the data were collected. Finally, temperature, salinity and sigma-t profile data were entered on a depth vs time graph. The resulting figures indicated the vertical movement of the isopleths over the associated time interval.

Recording current meter data can be treated in a variety of ways to bring out different characteristics of shelf circulation. The two methods selected for this report provide a quick summary of water movement past the current meter. By decomposing current vectors into speeds and directions, histograms can be constructed to show the distribution of these variables over the time interval the current meter was in place. Alternately, progressive vector diagrams, constructed by plotting current vectors in a head-to-tail manner, show how water moved past the study site, but may also be used to infer the general characteristics of the subsurface circulation along a given part of the coastline. More sophisticated analytical techniques provide a logical follow-up to this elementary analysis, but they are not necessarily required in a baseline monitoring study with an emphasis on the biology and chemistry of STOCS waters.

RESULTS

Results of the 1977 hydrographic and physical oceanographic sampling program are divided into two sections, one dealing with STD data and the computed hydrographic variables, and the other recording current meter data. Hydrographic data are presented chronologically, followed by a sub-section in which the year's data are combined to summarize seasonal and annual variations. Taken together, the data may be used to characterize the hydrographic climate of the STOCS waters. The direct current measurements help explain both the spatial distribution of hydrographic variables, and the temporal representativeness of the patterns measured during monthly and seasonal cruises.

Winter Seasonal Cruise Data

Transect I (January 18-22; February 21-22, 1977)

The temperature cross-section constructed for Transect I (Figure 2.3) must be interpreted with caution as it is patched together between Stations 2/I and 5/I. The inner half of the shelf was sampled on 21 January and the outer half was completed a month later on 21 February. Strongest horizontal temperature gradients were found inside Station 2/I. Vertical stratification, particularly strong at Station 1/I, was absent at Station 2/I. Over the entire shelf, water was quite homogeneous both horizontally and vertically. There was some indication that water between 17 and 18°C was moving up the outer shelf and rising to the surface just outside Station 2/I.

Salinity variations along Transect I (Figure 2.4) were restricted to within Station 2/I. This pattern was similar to that shown in Figure 2.3, with horizontal gradients occurring between Stations 1/I and 2/I, and vertical stratification strongest at Station 1/I. It was of interest to note that lowest salinities were found approximately 10 kilometers offshore.

Transect II (January 11-12 and February 21-22)

Temperatures along Transect II (Figure 2.5) were obtained a month

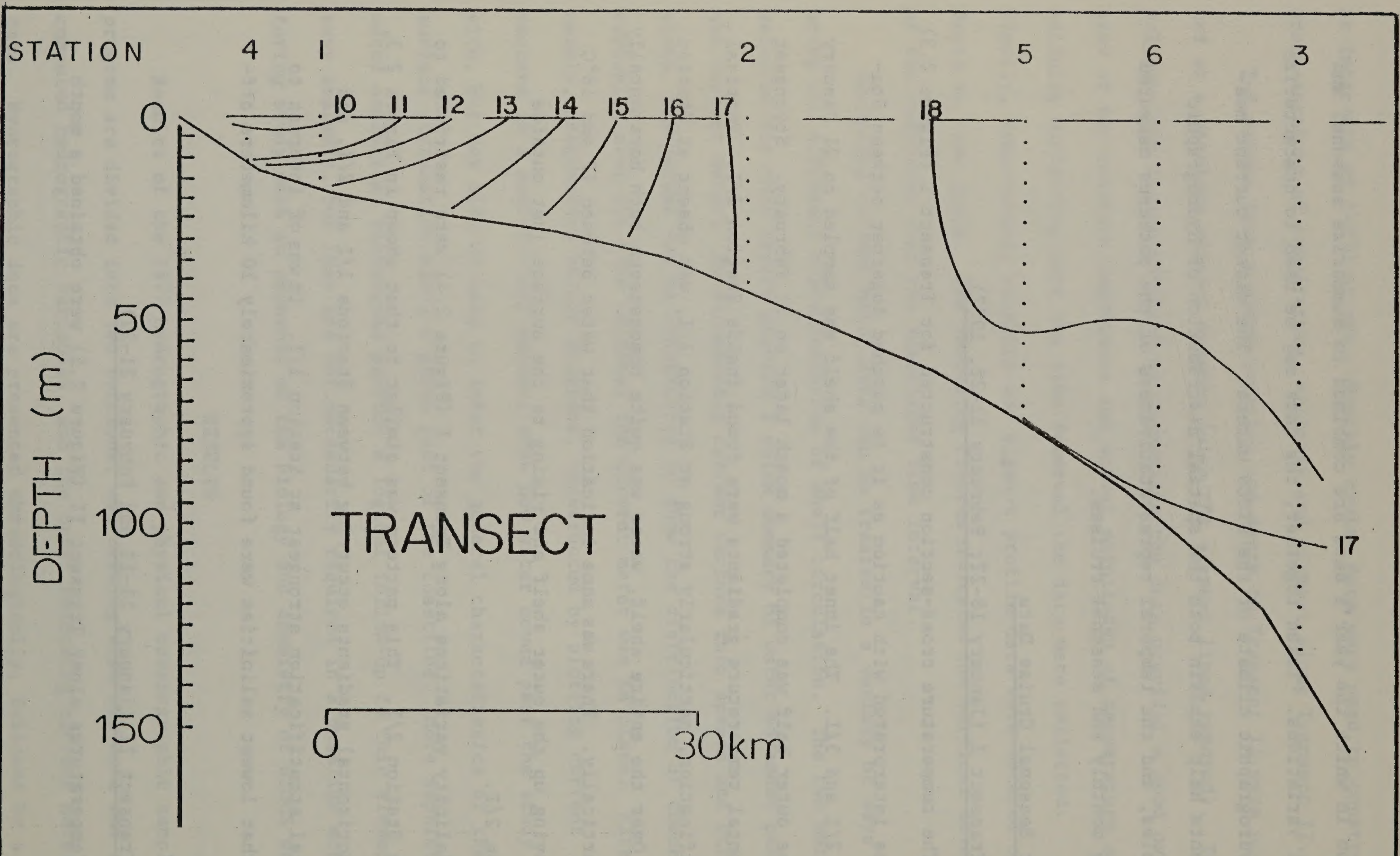

Figure 2.3 Winter Temperatures (°C) for 21 January (Stations 4, 1 and 2) and 21 February 1977.

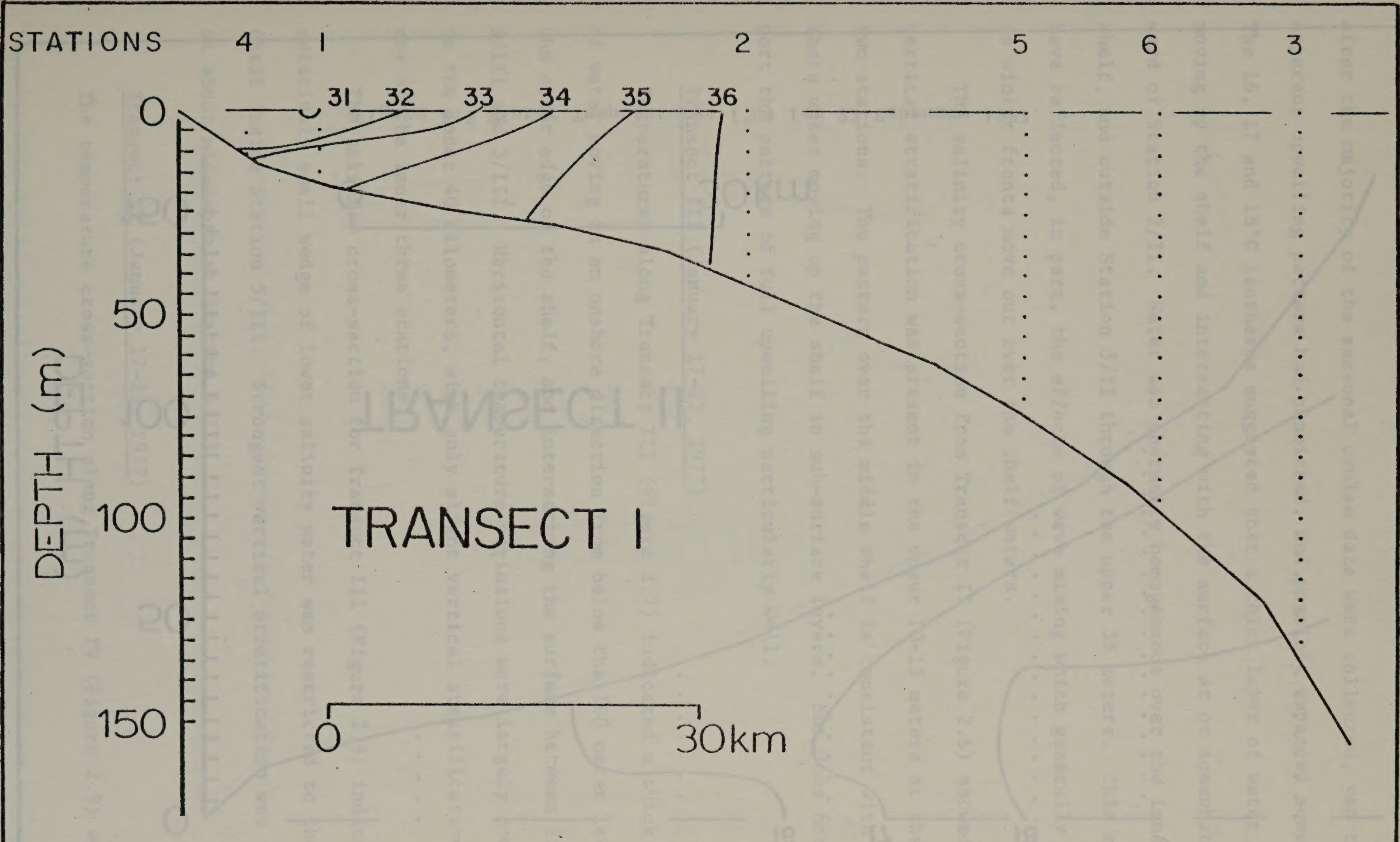

Figure 2.4 Winter Salinities (ppt) for 21 January (Stations 4, 1 and 2) and 21 February 1977.

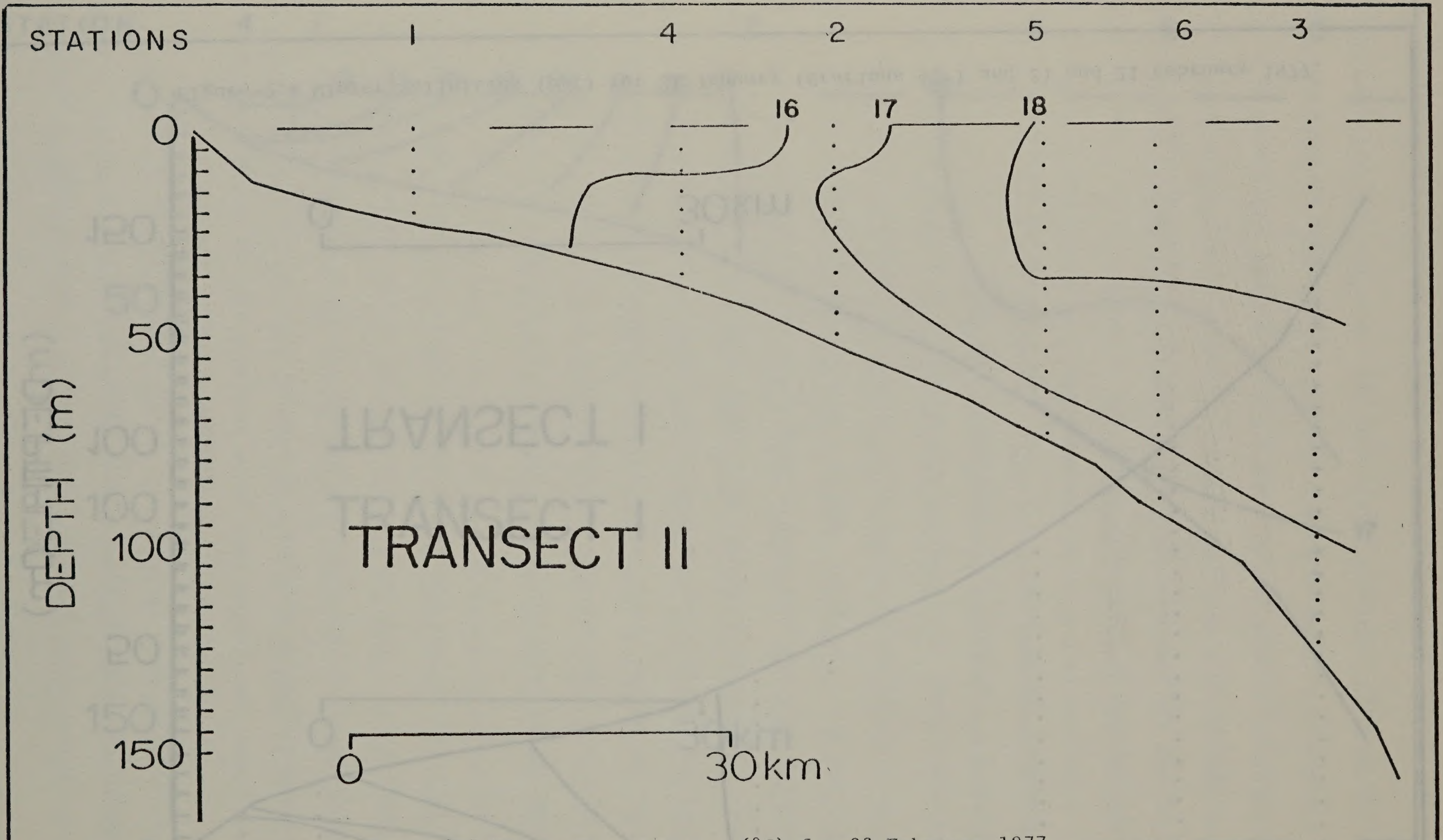

Figure 2.5 Winter Temperatures (°C) for 22 February 1977.

after the majority of the seasonal cruise data were collected, yet the apparent upwelling pattern had persisted, and possibly expanded somewhat. The 16, 17 and 18°C isotherms suggested that a thick layer of water was moving up the shelf and intersecting with the surface at or somewhat seaward of Station 2/II. Water was thermally homogeneous over the inner shelf, and outside Station 5/II through the upper 35 meters. This may have reflected, in part, the effects of wave mixing which generally occurs as winter fronts move out over the shelf waters.

The salinity cross-section from Transect II (Figure 2.6) showed that vertical stratification was present in the upper 10-15 meters at the inner two stations. The pattern over the middle shelf is consistent with offshore water moving up the shelf in sub-surface layers, but does not support the pattern of full upwelling particularly well.

Transect III (January 17-22, 1977)

Temperatures along Transect III (Figure 2.7) indicated a thick layer of water moving in an onshore direction from below the 100 meter level at the outer edge of the shelf, and intersecting the surface between Stations 5/III and 3/III. Horizontal temperature variations were largely confined to the inner 40 kilometers, with only slight vertical stratification at any of the inner three stations.

The salinity cross-section for Transect III (Figure 2.8) indicates a relatively small wedge of lower salinity water was restricted to the shelf inside Station 5/III. Strongest vertical stratification was found at about mid-depth at Station 4/III.

Transect IV (January 17-22, 1977)

The temperature cross-section along Transect IV (Figure 2.9) was more

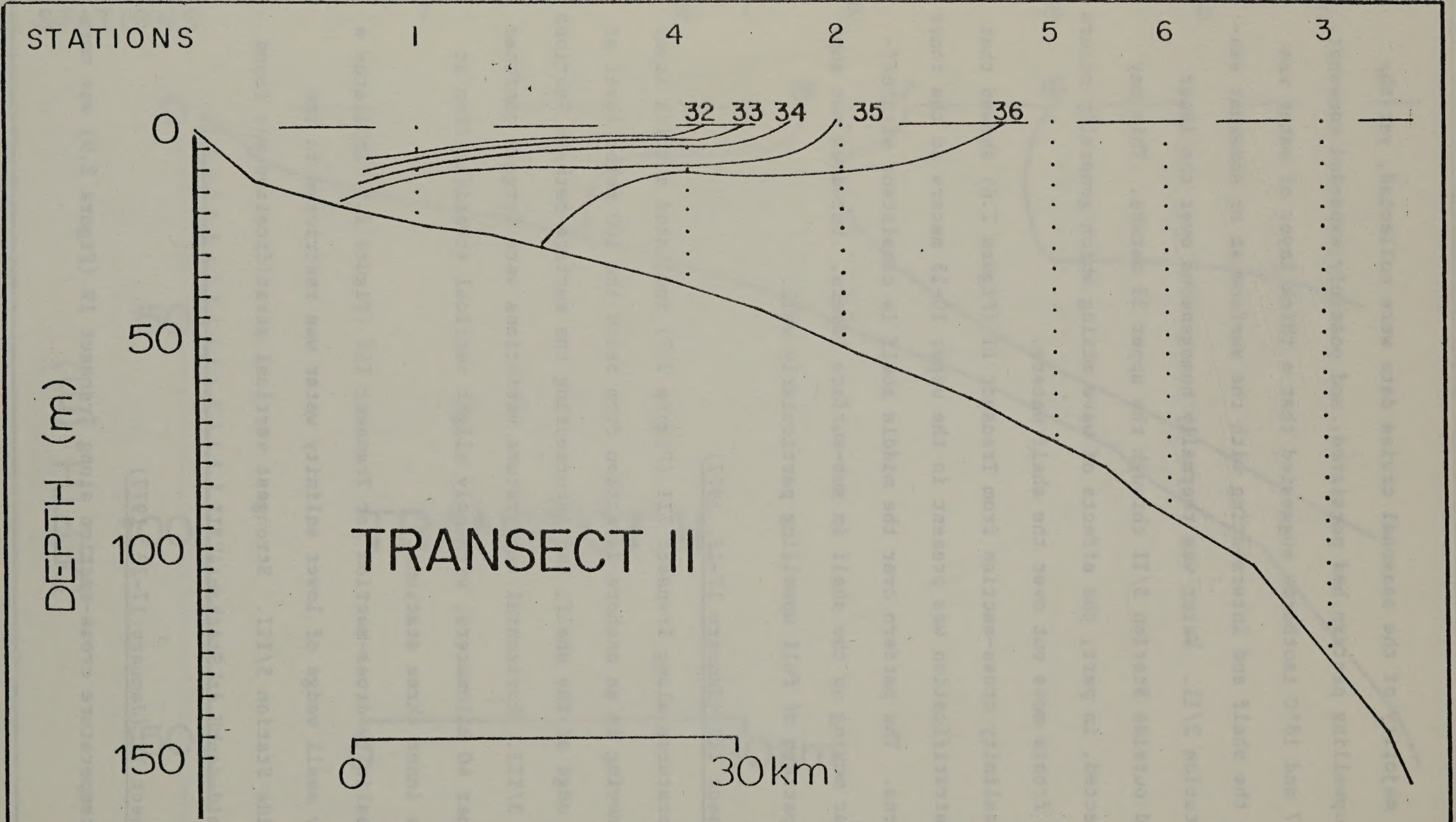

Figure 2.6 Winter Salinities (ppt) for 22 February 1977.

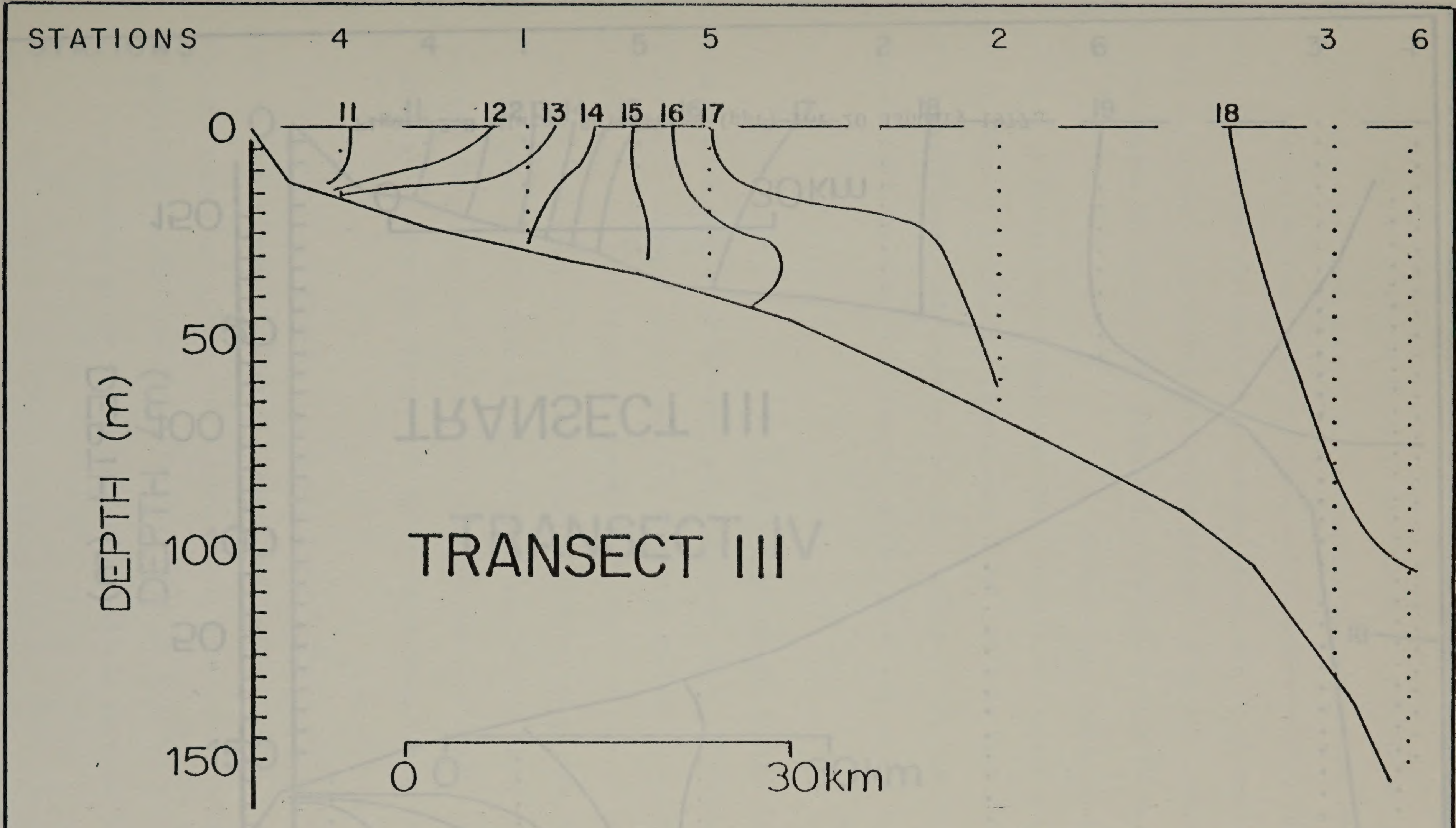

Figure 2.7 Winter Temperatures (°C) for 20 January 1977.

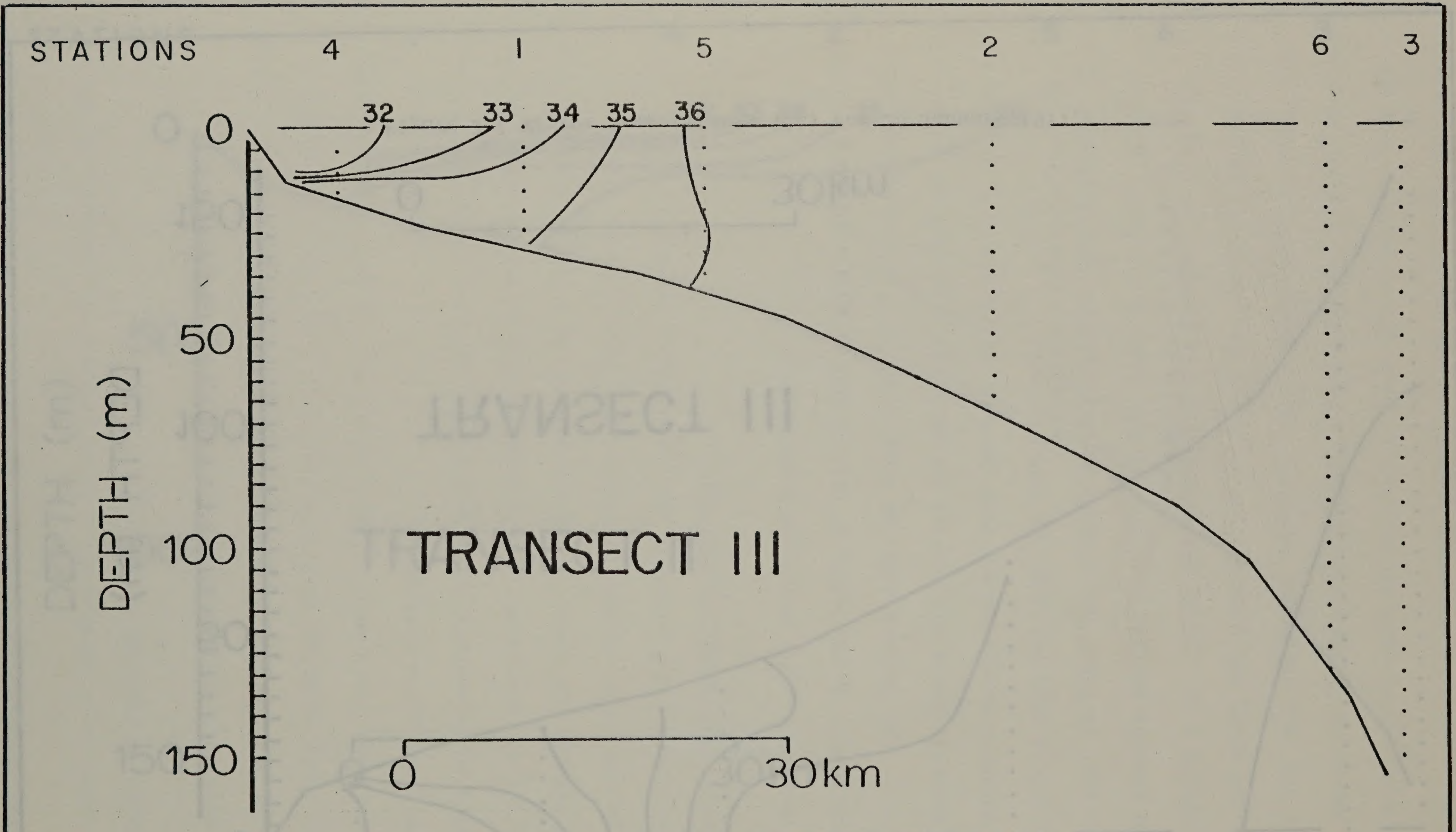

Figure 2.8 Winter Salinities (ppt) for 20 January 1977.

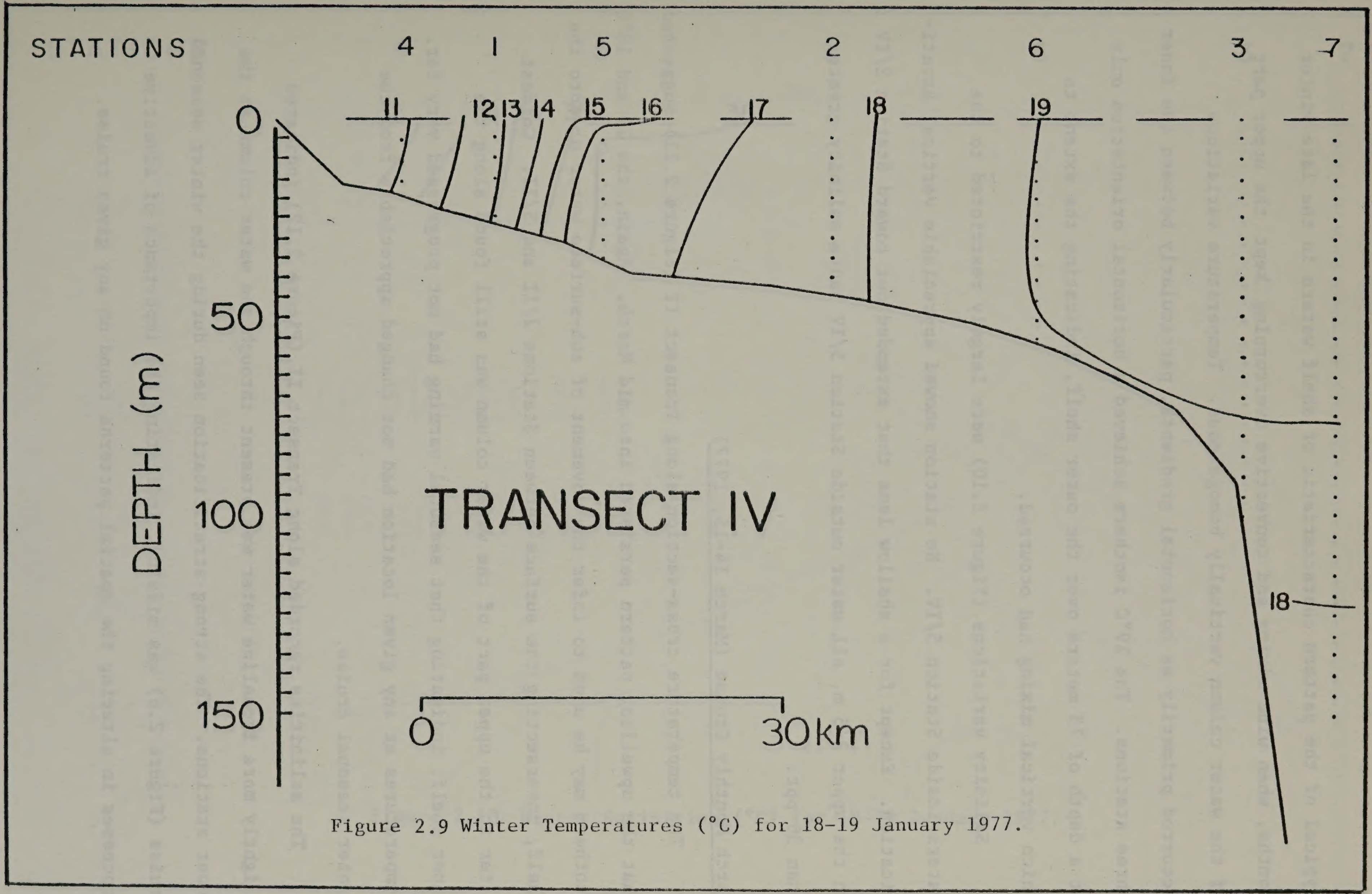

Figure 2.9 Winter Temperatures (°C) for 18-19 January 1977.

typical of the pattern characteristic of shelf waters in the late winter months, when wind mixing and convective overturning kept the upper part of the water column vertically homogeneous. Temperature variations occurred primarily as horizontal gradients, particularly between the inner three stations. The 19°C isotherm achieved a horizontal orientation only at a depth of 75 meters over the outer shelf, indicating the extent to which vertical mixing had occurred.

Salinity variations (Figure 2.10) were largely restricted to the waters inside Station 5/IV. No station showed appreciable vertical stratification. Except for a shallow lens that extended out toward Station 2/IV in the upper 3-5 m, all water outside Station 5/IV had a salinity greater than 36 ppt.

March Monthly Cruise (March 14-15, 1977)

The temperature cross-section along Transect II (Figure 2.11) suggested that the upwelling pattern persisted into mid March. Again, the 17 and 18°C isotherm may be used to infer the movement of sub-surface water up onto the shelf, intersecting the surface between Stations 2/II and 5/II. Coldest water in the upper part of the water column was still found along the inner shelf, indicating that seasonal warming had not progressed very far. Temperatures at any given location had not changed appreciably from the winter seasonal cruise.

The salinities recorded along Transect II (Figure 2.12) indicated slightly more isohaline water was present through the water column at the inner stations. The strong stratification seen during the winter seasonal cruise (Figure 2.6) was missing, indicating the importance of advective processes in altering the spatial patterns found on any given cruise.

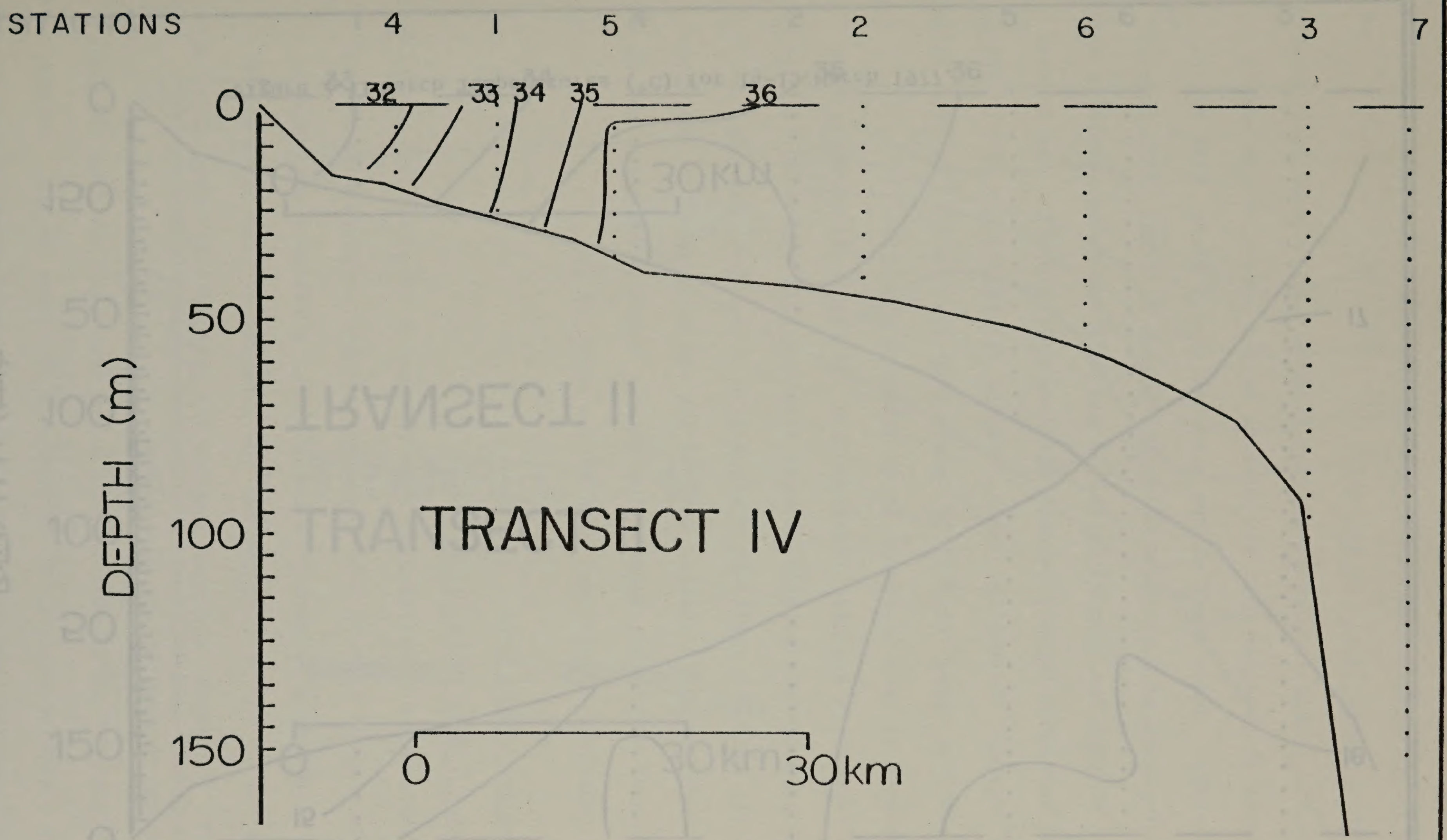

Figure 2.10 Winter Salinities (°C) for 18-19 January 1977.

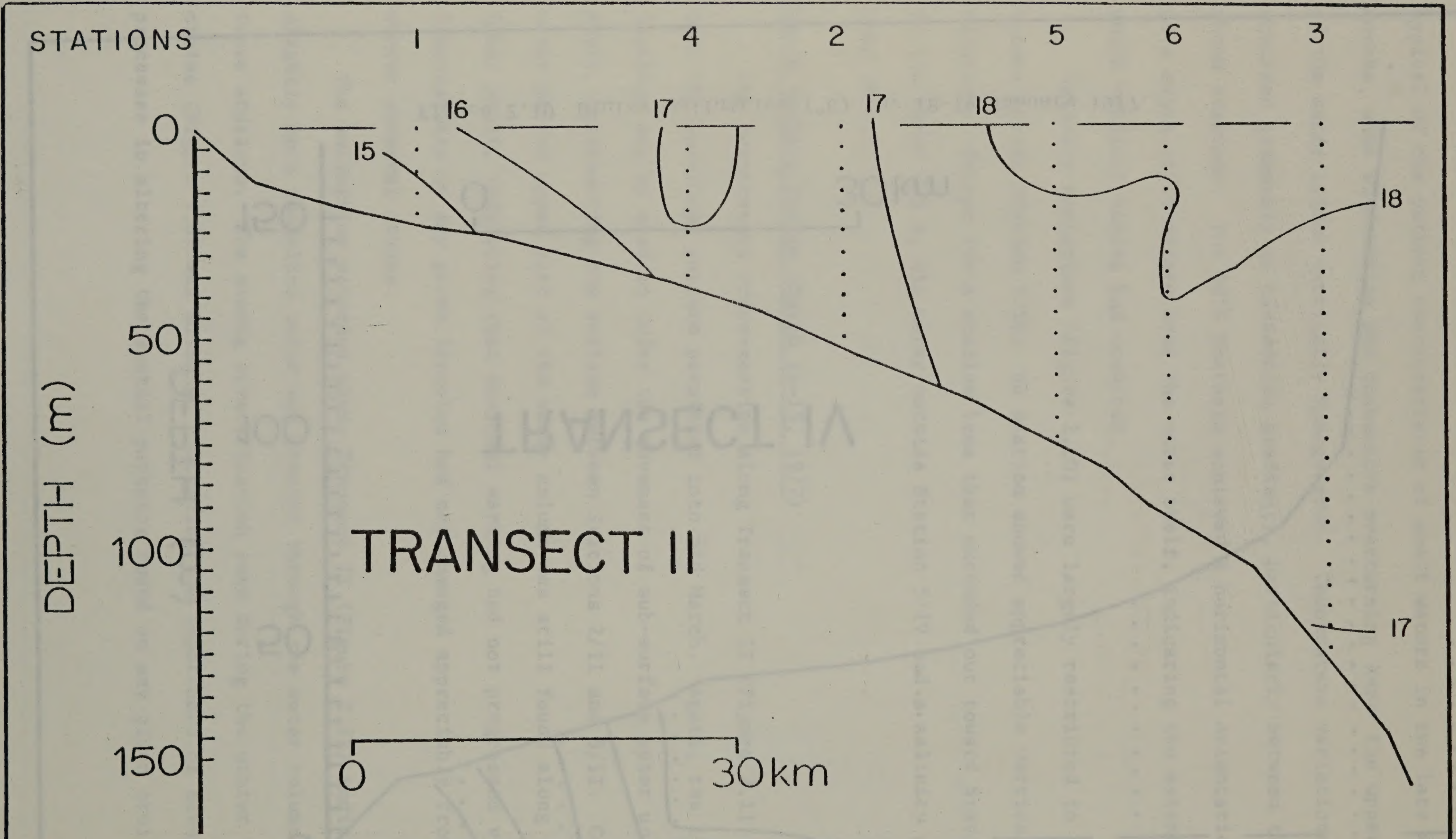

Figure 2.11 March Temperatures (°C) for 14-15 March 1977.

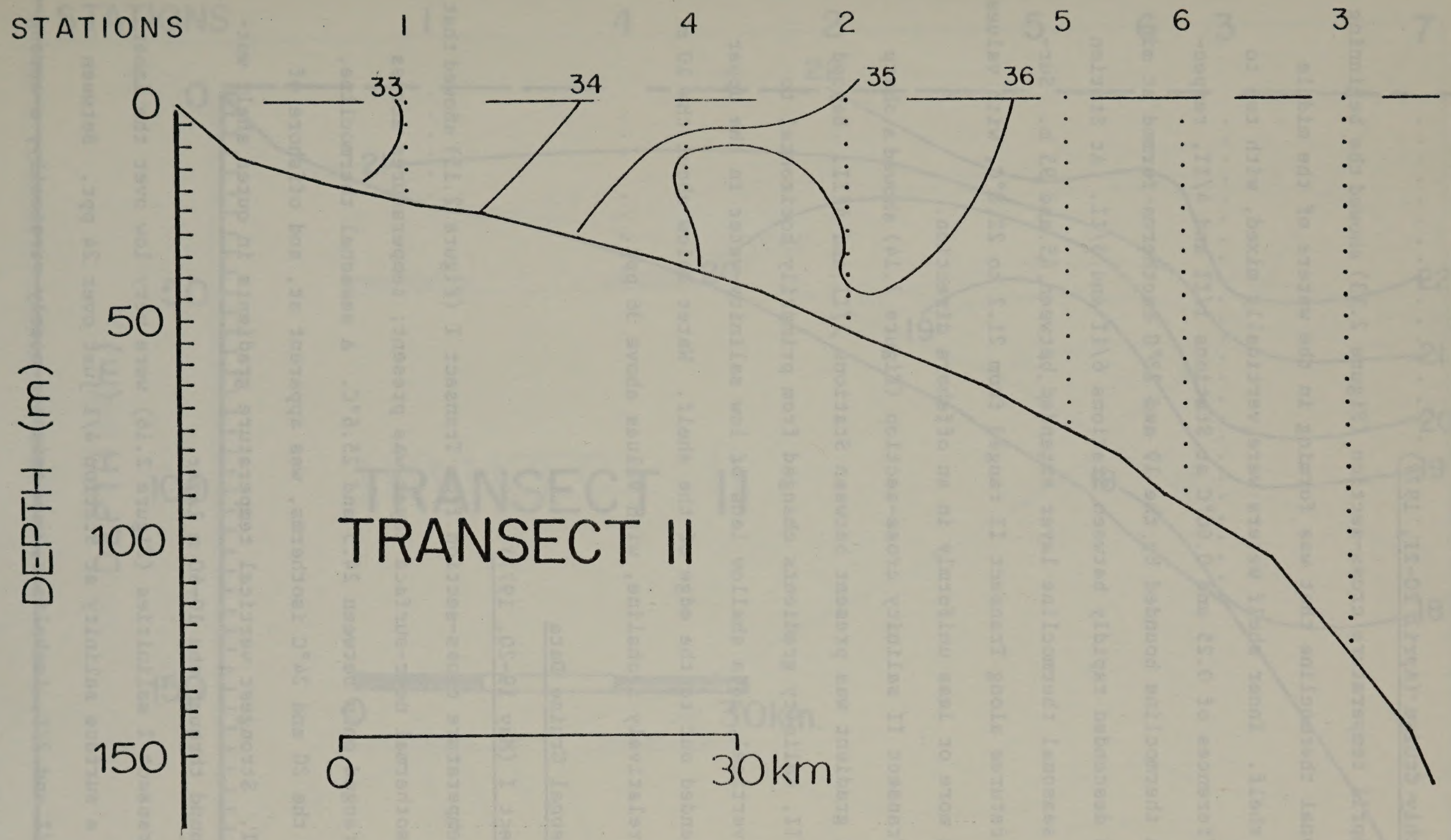

Figure 2.12 March Salinities (ppt) for 14-15 March 1977.

April Monthly Cruise (April 20-21, 1977)

The April temperature cross-section (Figure 2.13) showed the beginning of a seasonal thermocline that was forming in the waters of the middle and outer shelf. Inner shelf waters were vertically mixed, with top to bottom differences of 0.25 and 0.60°C at Stations 1/II and 4/II, respectively. A thermocline bounded by the 19 and 22°C isotherms formed at mid-depths and descended rapidly between Stations 6/II and 3/II. At Station 7/II, the seasonal thermocline layer extended between 45 and 95 m. Surface temperatures along Transect II ranged from 21.2 to 22.8°C, with values increasing more or less uniformly in an offshore direction.

The Transect II salinity cross-section (Figure 2.14) showed a sharp horizontal gradient was present between Stations 1/II and 4/II. Beyond Station 4/II, salinity gradients changed from primarily horizontal to primarily vertical, as a shallow lens of low salinity water in the upper 5-10 m extended out to the edge of the shelf. Water below about the 10 m level was relatively isohaline, with values above 36 ppt.

Spring Seasonal Cruise Data

Transect I (May 19-20, 1977)

The temperature cross-section from Transect I (Figure 2.15) showed that a nearly isothermal near-surface layer was present; temperatures across the shelf ranged only between 24.5 and 25.6°C. A seasonal thermocline, bounded by the 20 and 24°C isotherms, was apparent at, and offshore of Station 2/I. Strongest vertical temperature gradients in outer shelf waters were found through the 10-60 m layer.

The Transect I salinities (Figure 2.16) were very low over the inner shelf with a surface salinity at Station 4/I just over 24 ppt. Between Stations 4/I and 2/I, isohalines, were oriented nearly vertically; a strong

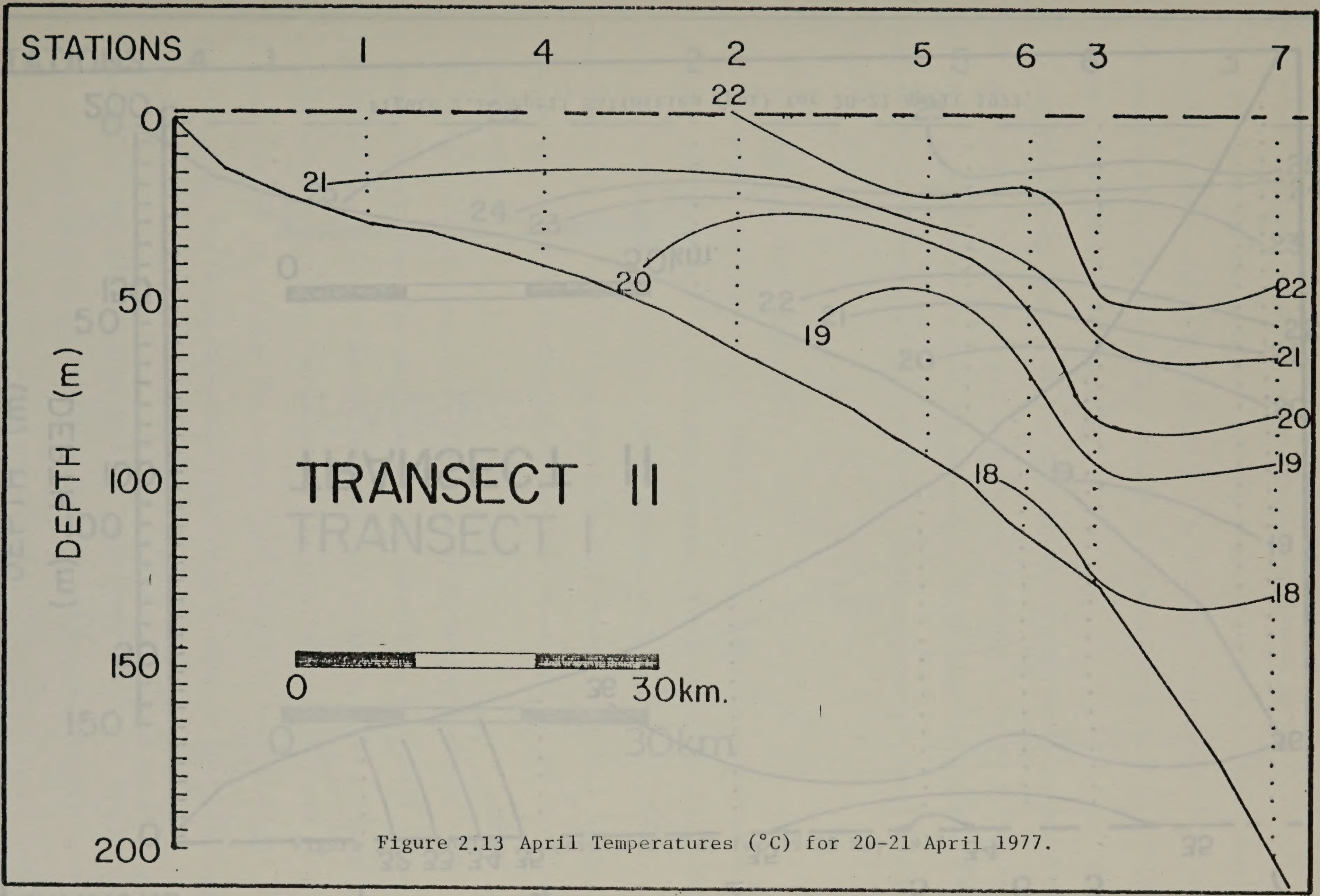

Figure 2.13 April Temperatures (°C) for 20-21 April 1977.

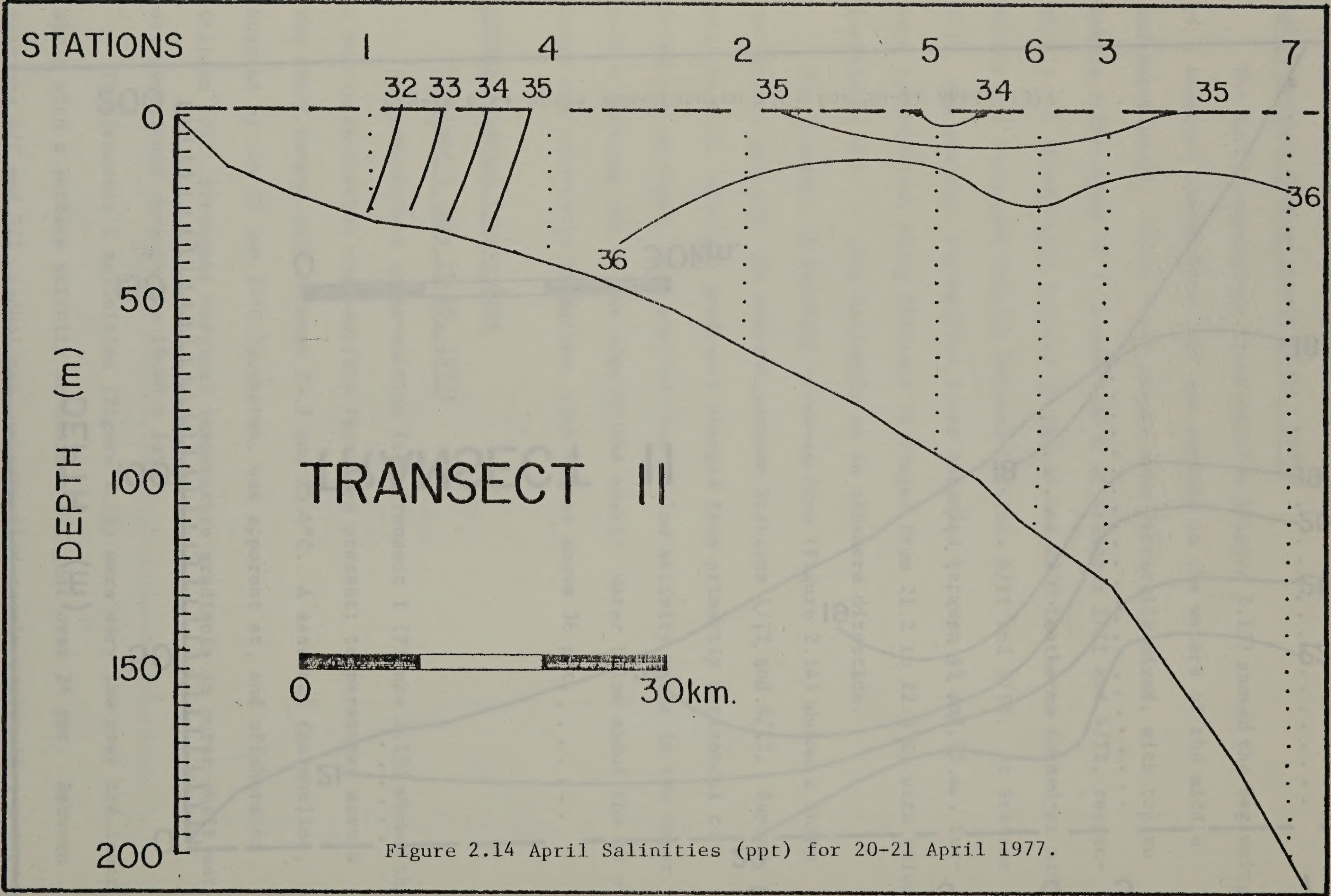

Figure 2.14 April Salinities (ppt) for 20-21 April 1977.

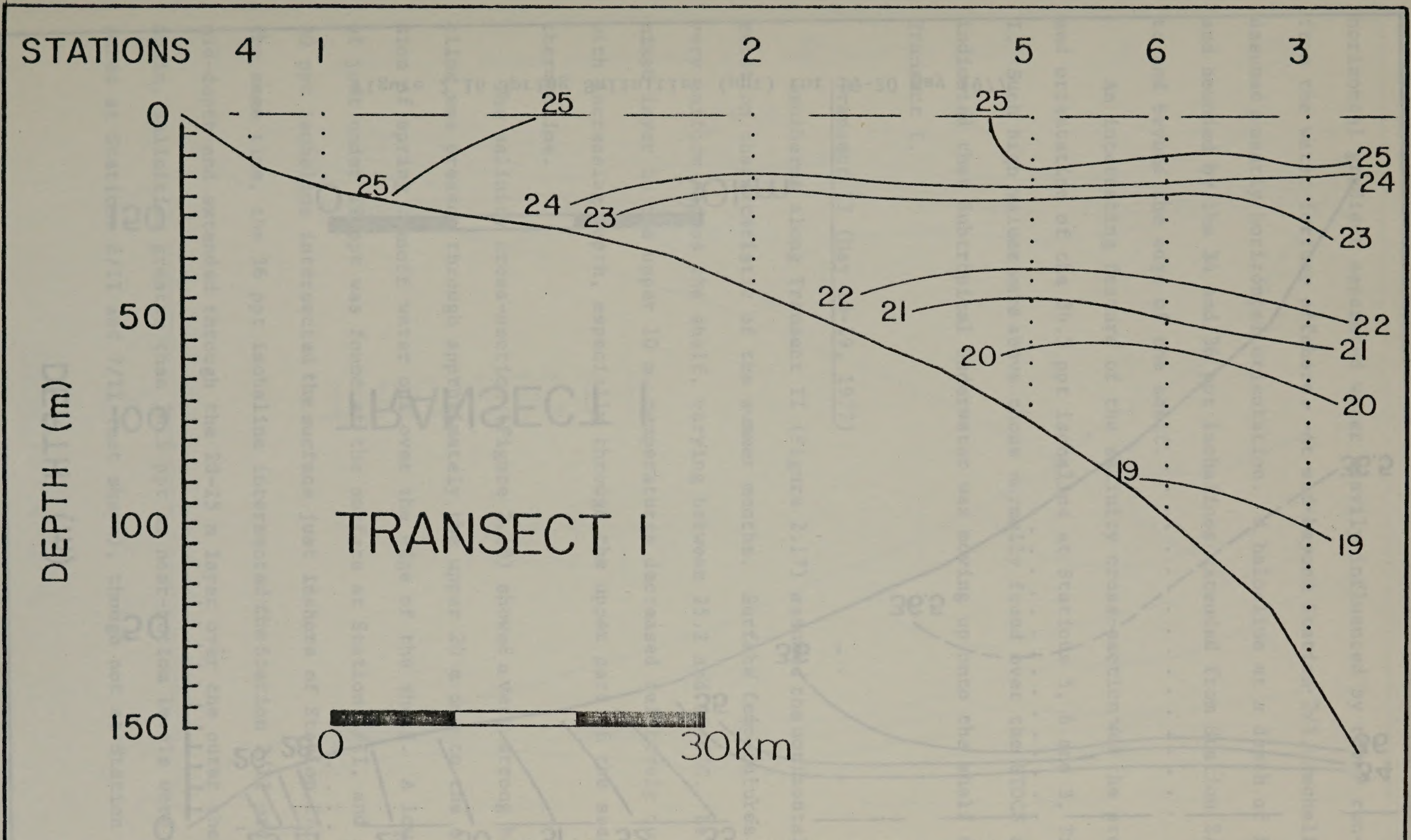

Figure 2.15 Spring Temperatures (°C) for 19-20 May 1977.

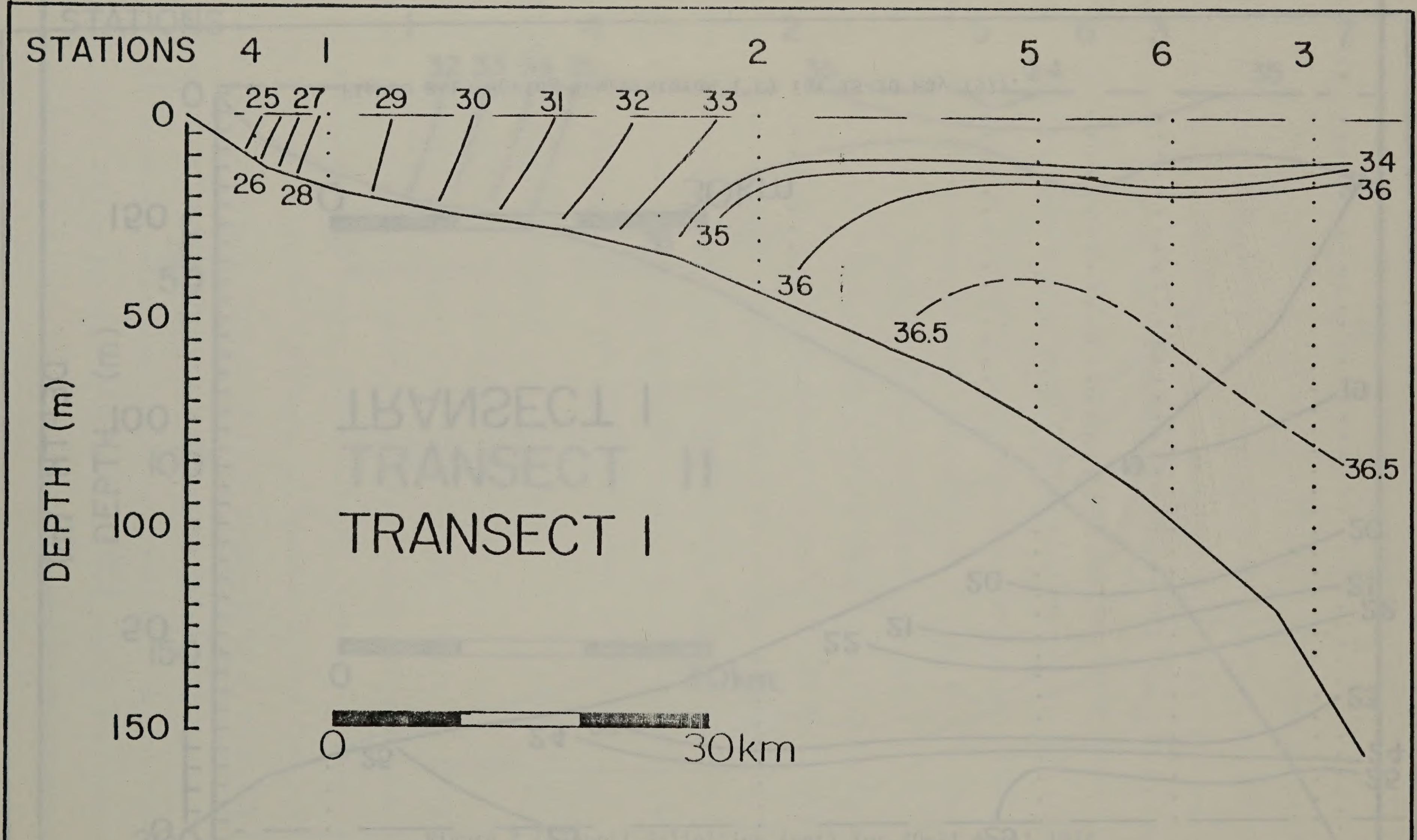

Figure 2.16 Spring Salinities (ppt) for 19-20 May 1977.

horizontal gradient separated water heavily influenced by river run-off from the water further offshore. At and beyond Station 2/I, isohalines assumed a nearly horizontal orientation. A halocline at a depth of 10-15 m and bounded by the 34 and 36 ppt isohalines extended from Station 2/I out to and beyond the edge of the shelf.

An interesting feature of the salinity cross-section was the presence and orientation of the 36.5 ppt isohaline at Stations 5, 6 and 3, Transect I. Such high values were above those normally found over the STOCS and indicated that Subtropical Underwater was moving up onto the shelf along Transect I.

Transect II (May 18-19, 1977)

Isotherms along Transect II (Figure 2.17) assumed the horizontal orientation characteristic of the summer months. Surface temperatures were very uniform across the shelf, varying between 25.2 and 25.6°C. Below a mixed layer in the upper 10 m, temperatures decreased relatively quickly with increasing depth, especially through the upper part of the seasonal thermocline.

The salinity cross-section (Figure 2.18) showed a very strong halocline was present through approximately the upper 20 m due to the extension of spring runoff water out over the edge of the shelf. A low value of just under 29 ppt was found at the surface at Station 1/II, and the 30 ppt isohaline intersected the surface just inshore of Station 2/II. At the same time, the 36 ppt isohaline intersected the Station 2/II profile at mid-depth and extended through the 20-25 m layer over the outer shelf. Again, salinities greater than 36.5 ppt at near-bottom levels were measured at Stations 6/II and 7/II (not shown), though not at Station 3/II.

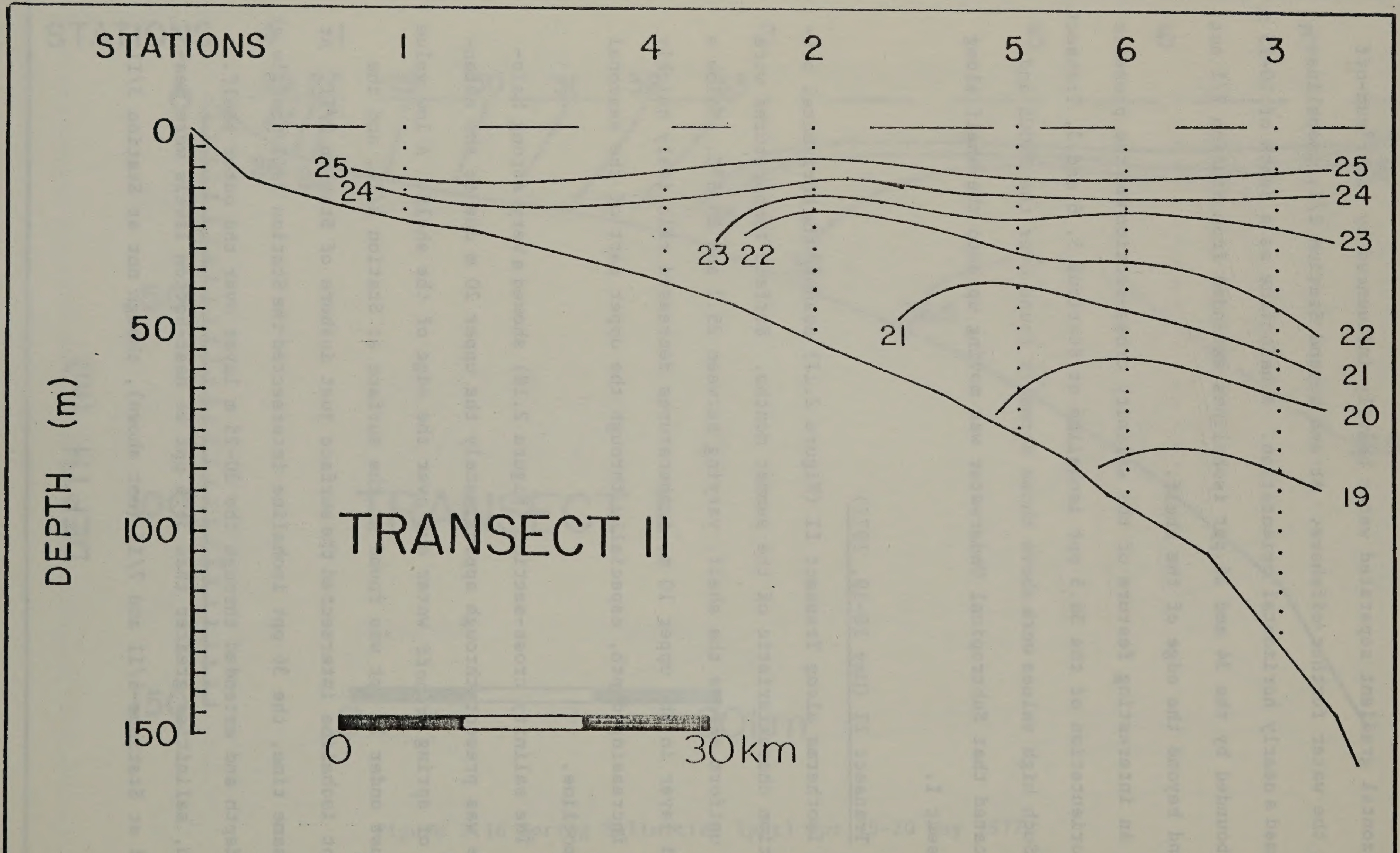

Figure 2.17 Spring Temperatures (°C) for 18-19 May 1977.

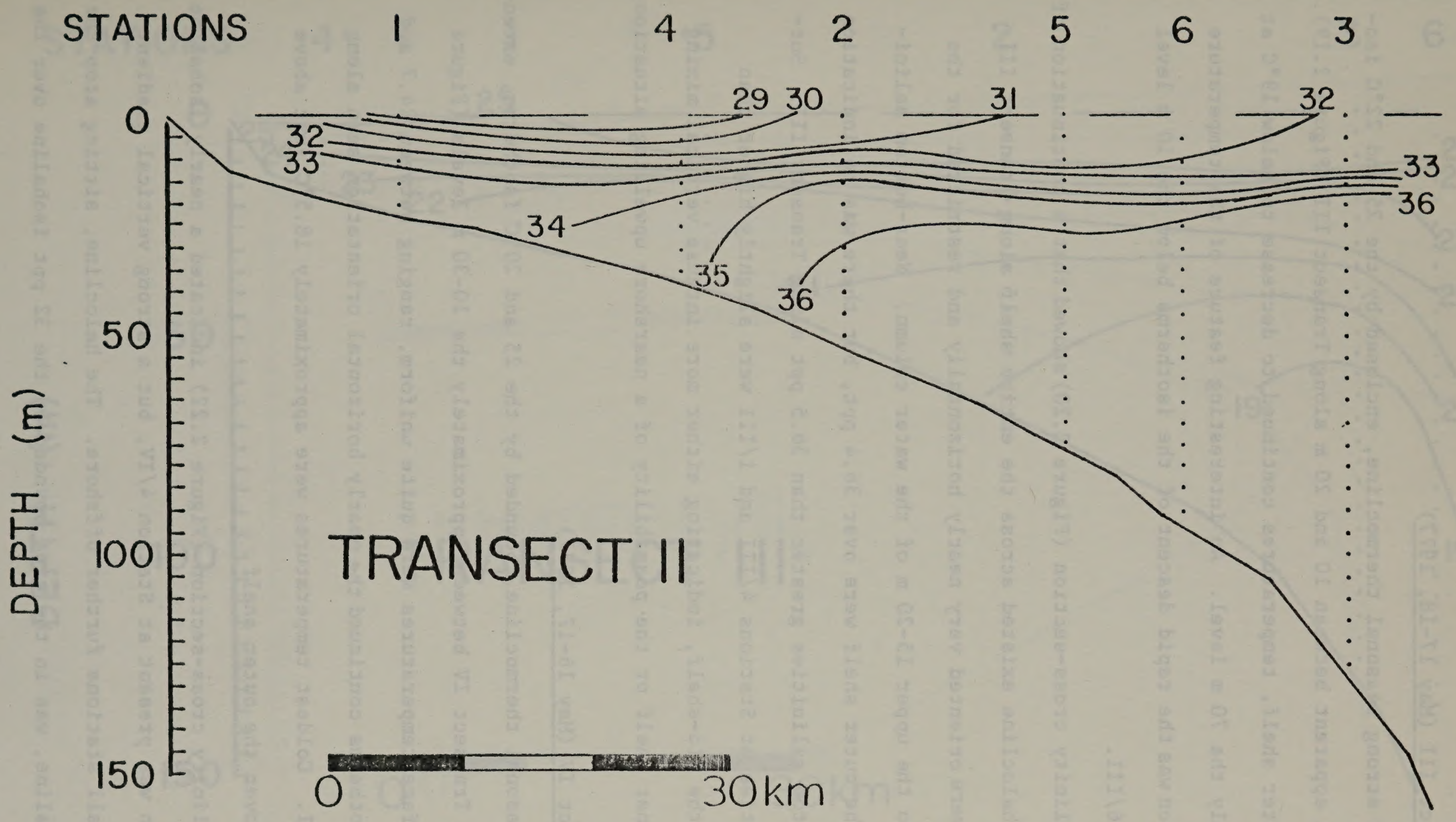

Figure 2.18 Spring Salinities (ppt) for 18-19 May 1977.

Transect III (May 17-18, 1977)

A very strong seasonal thermocline, enclosed by the 25 and 22°C isotherms, was apparent between 10 and 20 m along Transect III (Figure 2.19). Over the outer shelf, temperatures continued to decrease to below 19°C at approximately the 70 m level. An interesting feature of the temperature cross-section was the rapid descent of the isotherms below the 10 m level at Station 6/III.

The salinity cross-section (Figure 2.20) showed that a continuation of the strong halocline existed across the entire shelf along Transect III. Isohalines were oriented very nearly horizontally and restricted for the most part to the upper 15-20 m of the water column. Near-bottom salinities over the outer shelf were over 36.4 ppt, but there was no indication of near-bottom salinities greater than 36.5 ppt along Transect III. Surface salinities at Stations 4/III and 1/III were slightly higher than those over the mid-shelf, indicating either more intense vertical mixing over the inner shelf or the possibility of a nearshore upwelling situation.

Transect IV (May 16-17, 1977)

The seasonal thermocline, bounded by the 25 and 20°C isotherms, extended through Transect IV between approximately the 10-30 m levels (Figure 2.21). Surface temperatures were quite uniform, ranging between 24.7 and 25.2°C. Isotherms continued the nearly horizontal orientation seen along Transect III. Coldest temperatures were approximately 18.5°C just above the bottom over the outer shelf.

The salinity cross-section (Figure 2.22) indicated a nearly isohaline water column was present at Station 4/IV, but a strong vertical gradient existed at all stations further offshore. The halocline, sitting atop the 36 ppt isohaline, was in the area bounded by the 32 ppt isohaline over the

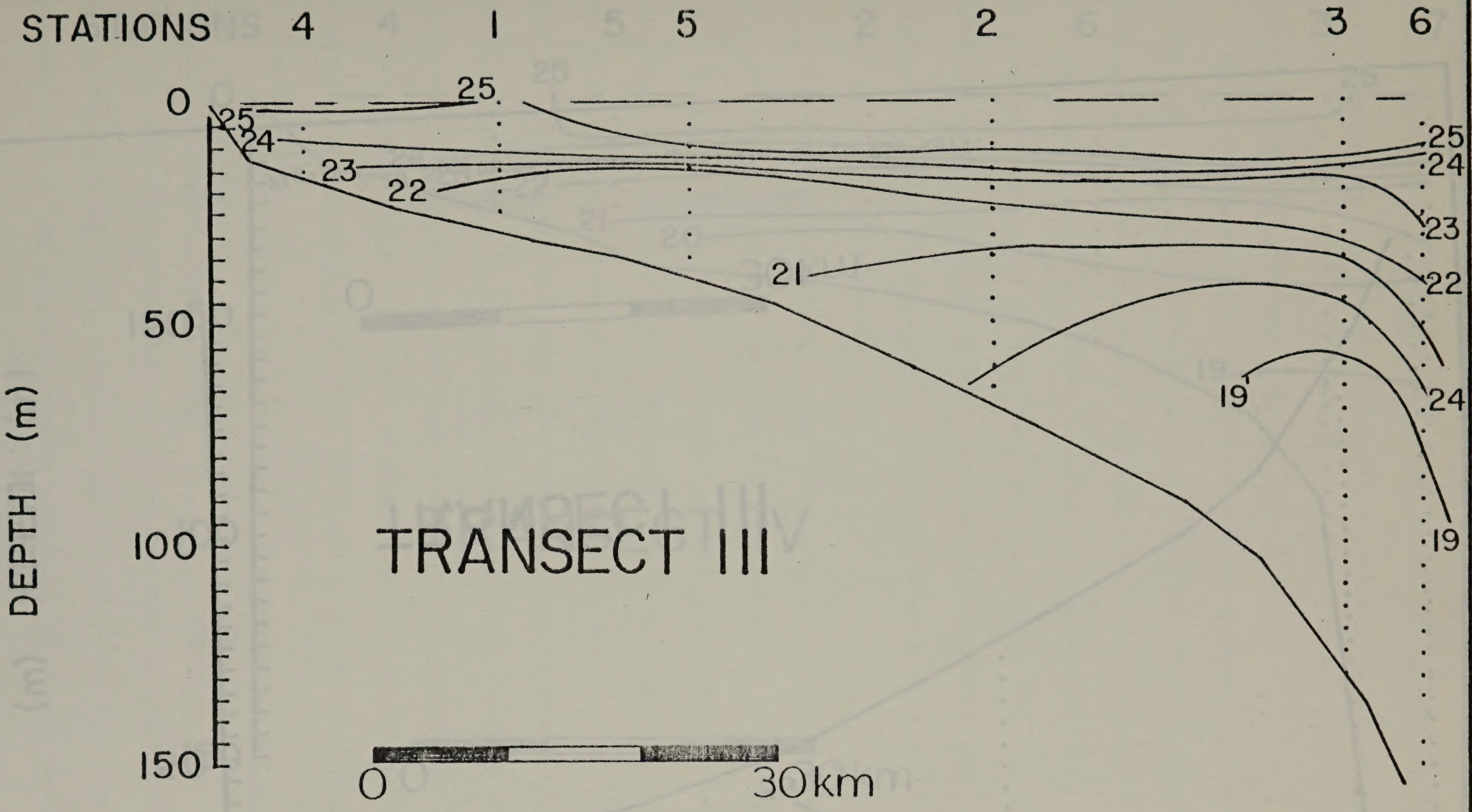

Figure 2.19 Spring Temperatures (°C) for 17-18 May 1977.

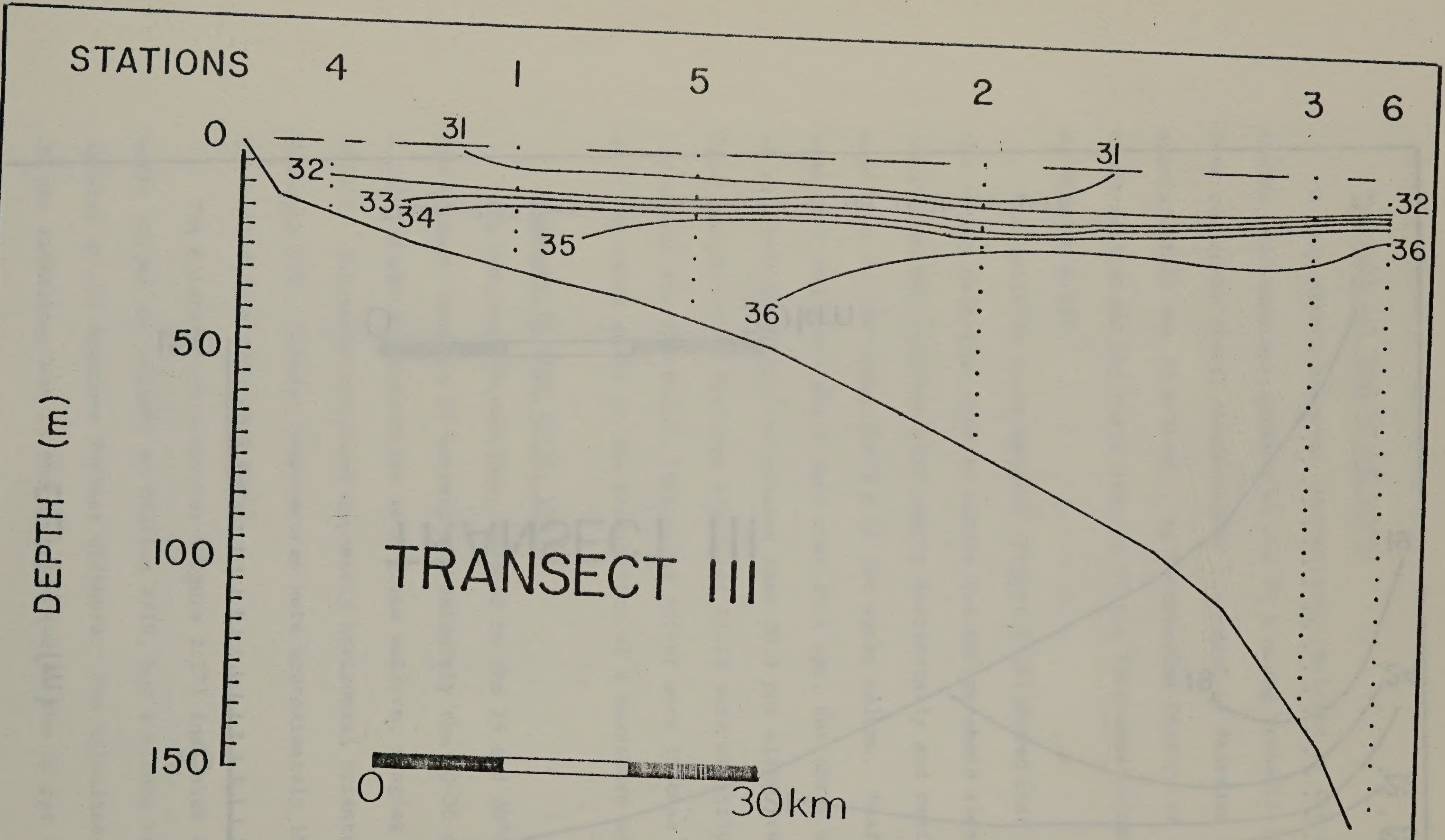

Figure 2.20 Spring Salinities (ppt) for 17-18 May 1977.

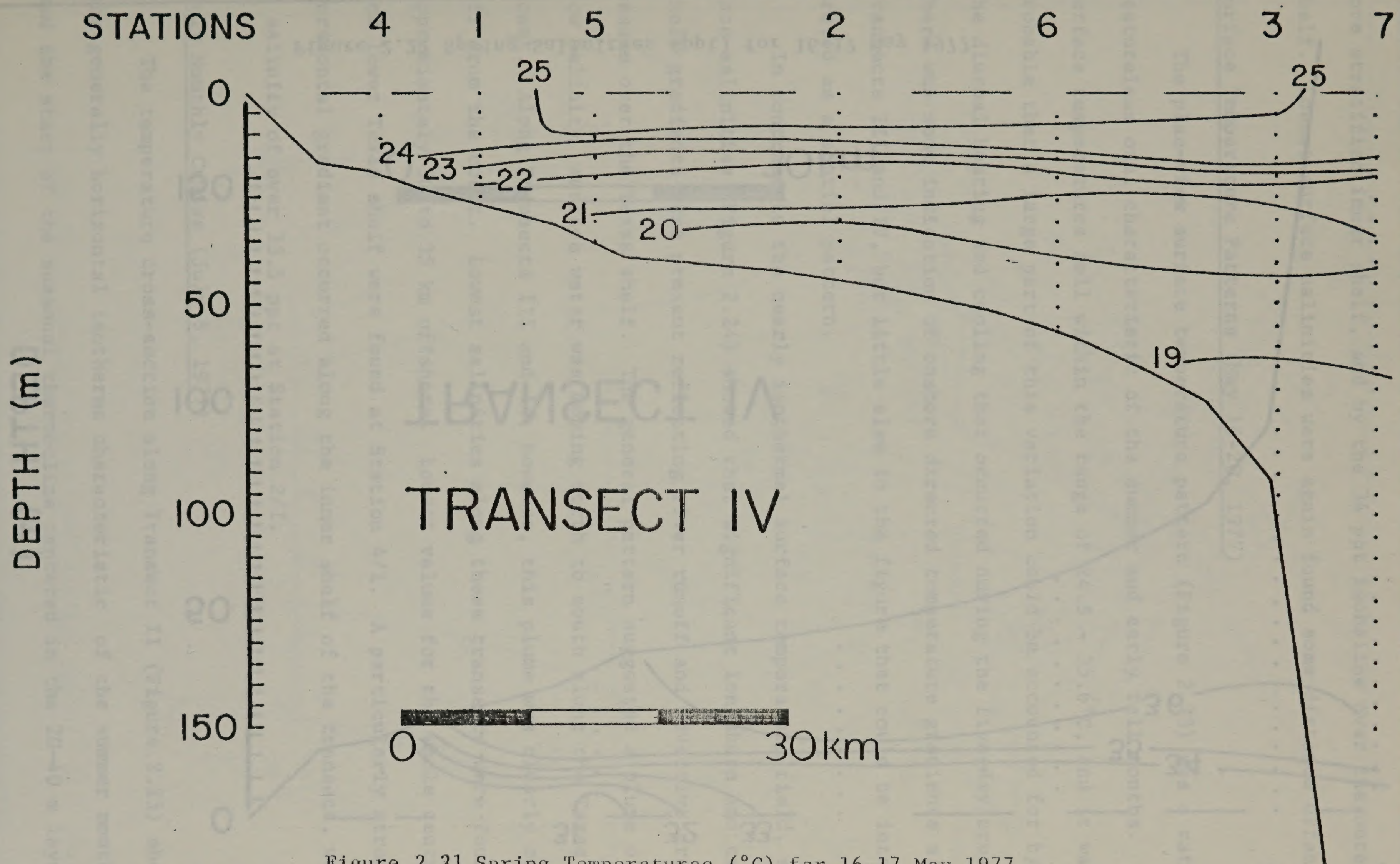

Figure 2.21 Spring Temperatures (°C) for 16-17 May 1977.

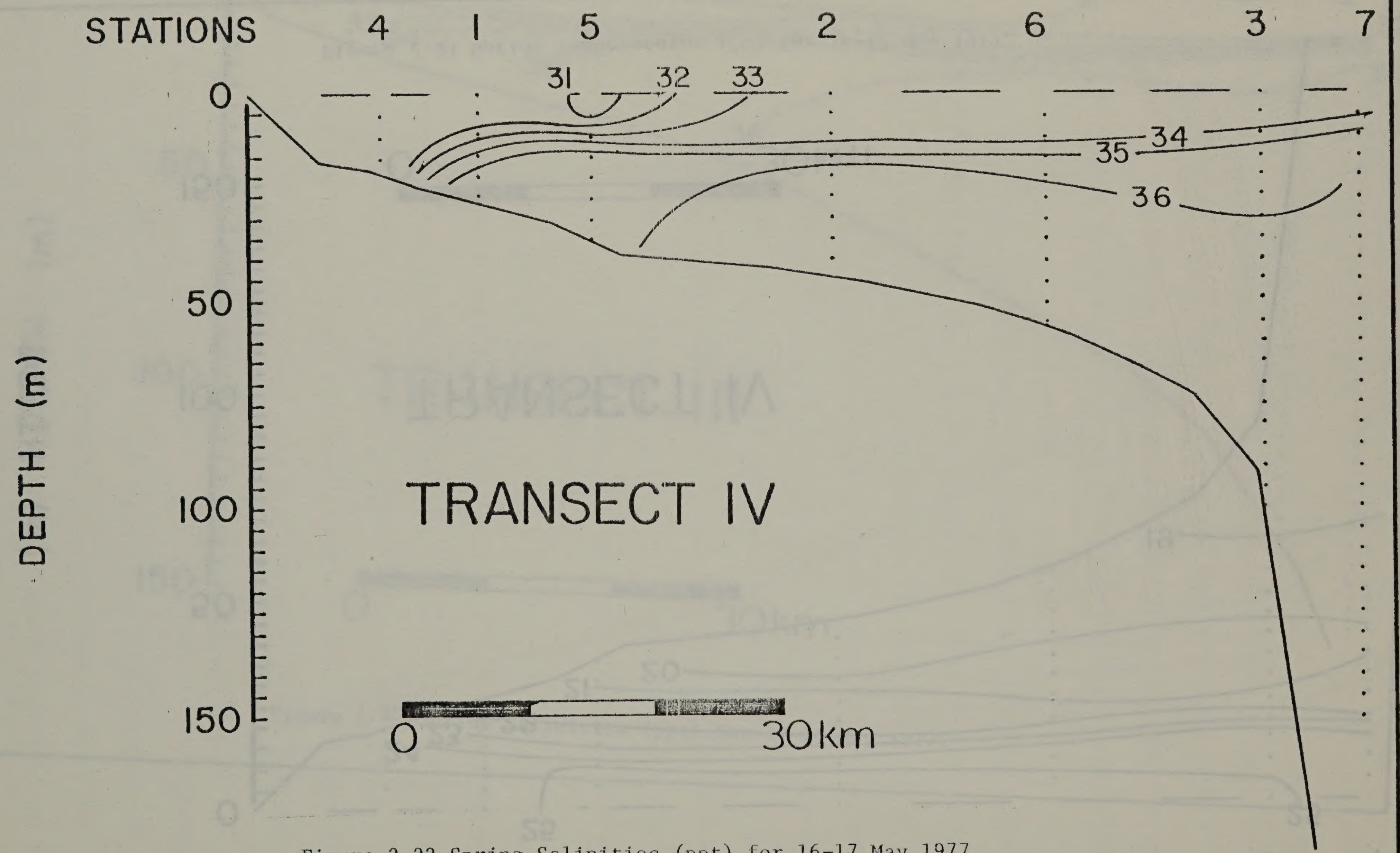

Figure 2.22 Spring Salinities (ppt) for 16-17 May 1977.

more stratified inner shelf, and by the 34 ppt isohaline over the outer shelf. Lowest surface salinities were again found some distance offshore.

Surface Temperature Patterns (May 16-20, 1977)

The plan-view surface temperature pattern (Figure 2.23) was a rather featureless one, characteristic of the summer and early fall months. All surface temperatures fell within the range of 24.5 - 25.6°C, and it was probable that a large part of this variation could be accounted for by the diurnal heating and cooling that occurred during the five-day cruise. There was some indication of onshore directed temperature gradients along Transects III and IV, but little else in the figure that could be interpreted as a spatial pattern.

In contrast to the nearly isothermal surface temperature field, surface salinities (Figure 2.24) showed that significant longshore and cross-shelf gradients were present reflecting river runoff and advective processes over the Texas shelf. The general pattern suggested a plume of low salinity surface water was moving north to south along the Texas coast. Along Transects III and IV, however, this plume was clearly cut off from the coast. Lowest salinities along these transects were found approximately 30 to 35 km offshore. Lowest values for the whole central and lower Texas shelf were found at Station 4/I. A particularly strong horizontal gradient occurred along the inner shelf of the transect, with a salinity of over 33.5 ppt at Station 2/I.

July Monthly Cruise (July 6, 1977)

The temperature cross-section along Transect II (Figure 2.25) showed the generally horizontal isotherms characteristic of the summer months and the start of the seasonal thermocline centered in the 20-40 m layer.

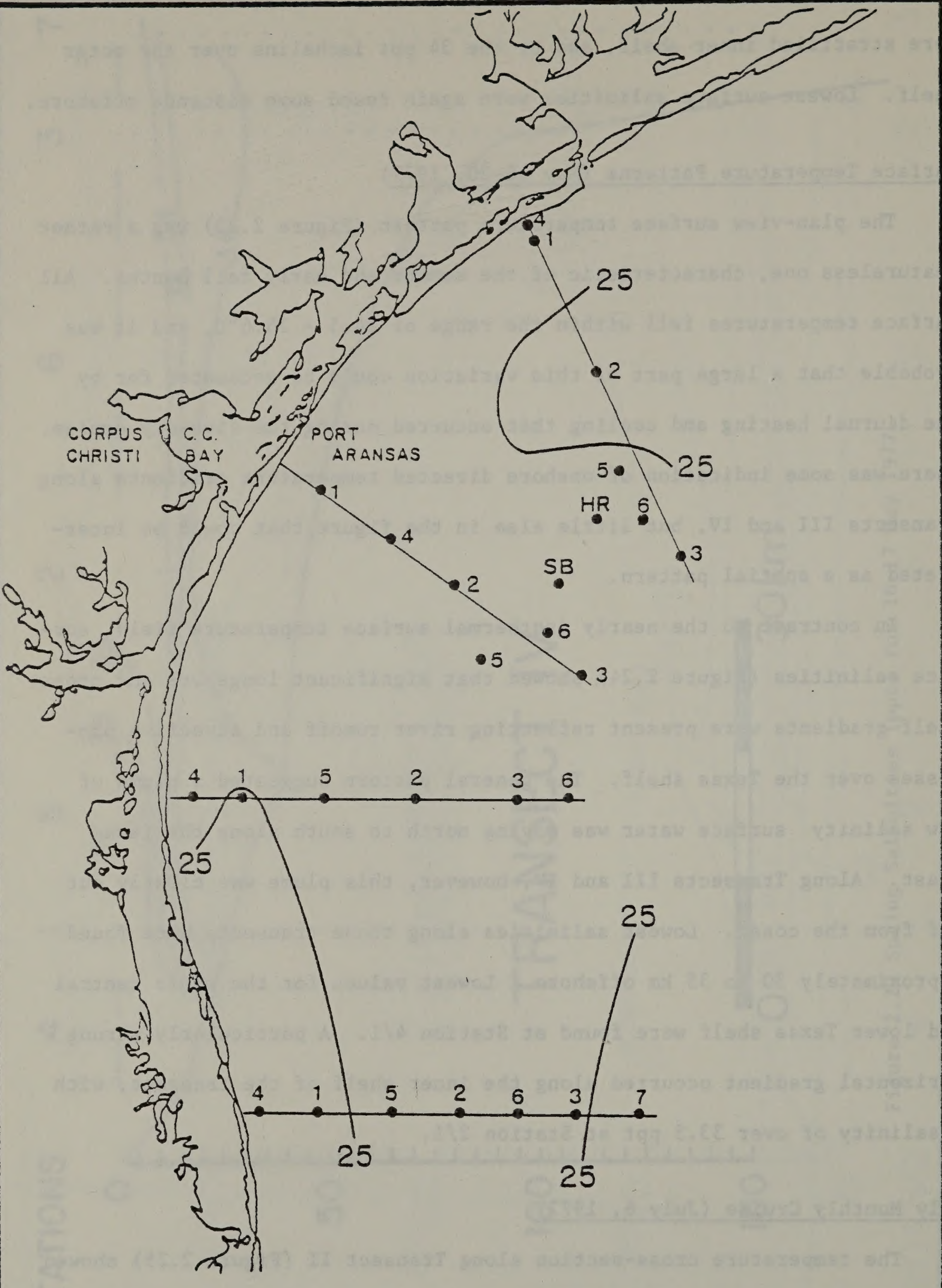

Figure 2.23 Spring Surface Temperature Pattern, 16-20 May 1977.

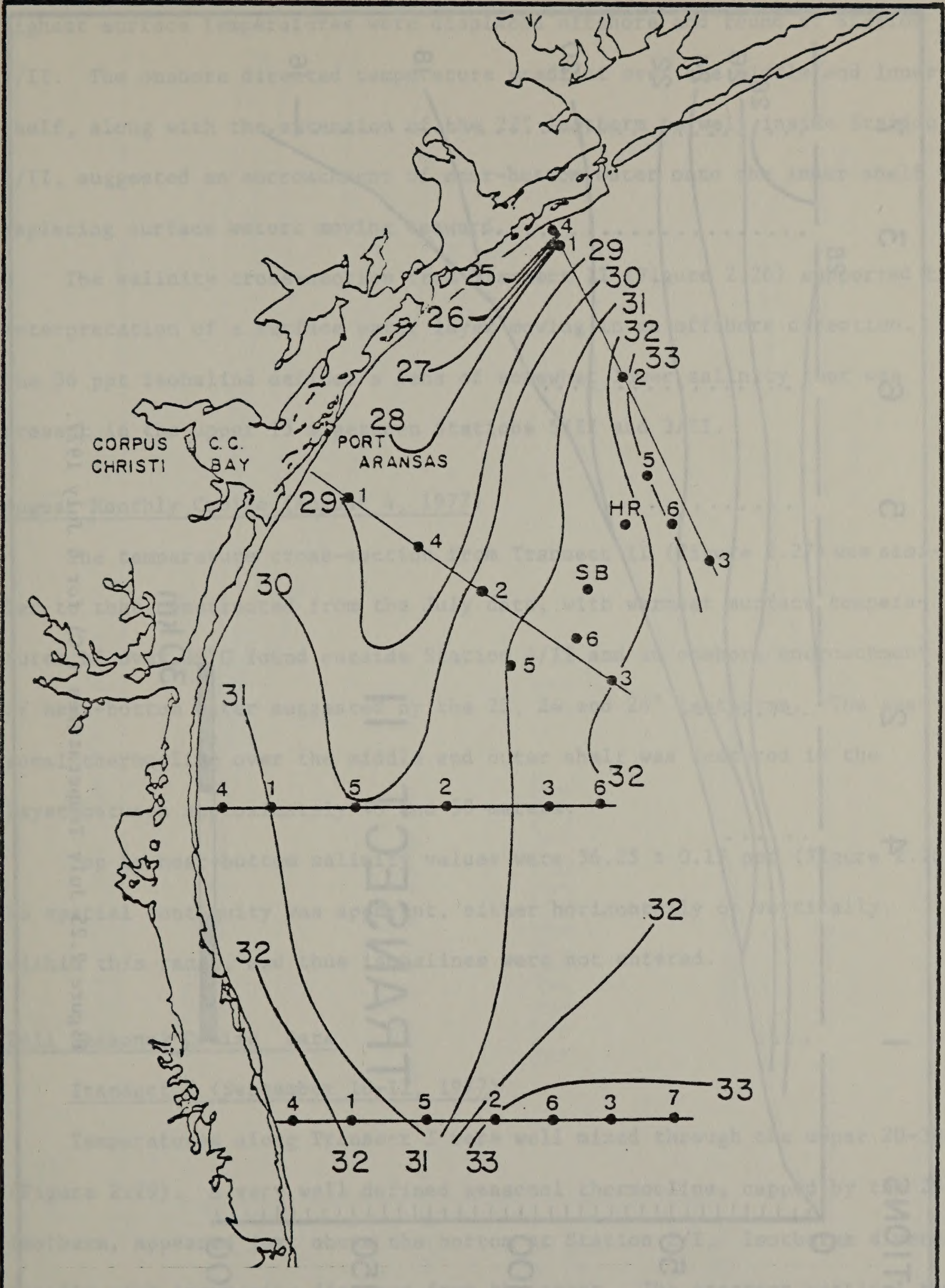

Figure 2.24 Spring Salinity Pattern, 16-20 May 1977.

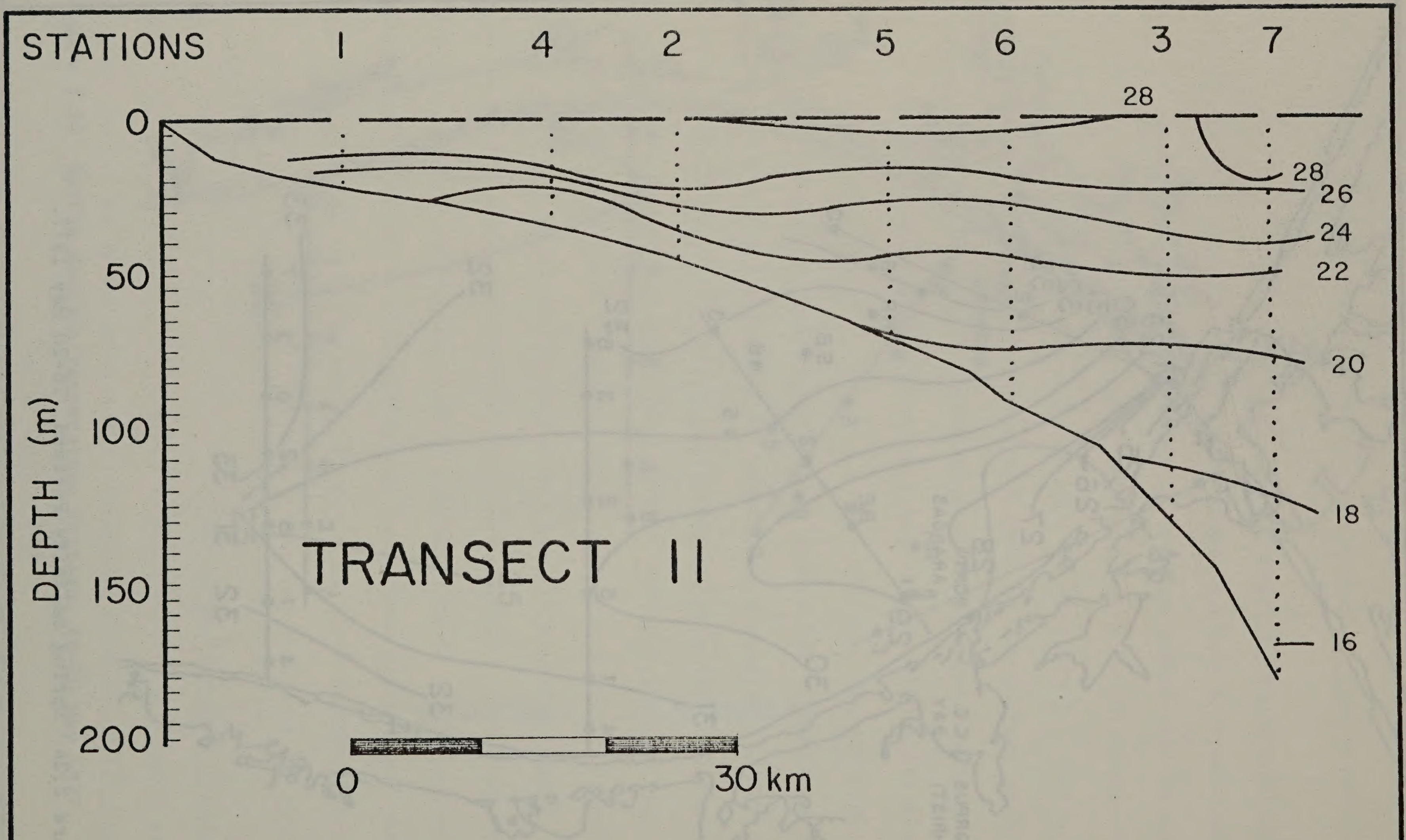

Figure 2.25 July Temperatures (°C) for 6 July 1977.

Highest surface temperatures were displaced offshore and found at Station 5/II. The onshore directed temperature gradient over the middle and inner shelf, along with the extension of the 22° isotherm to well inside Station 4/II, suggested an encroachment of near-bottom water onto the inner shelf replacing surface waters moving seaward.

The salinity cross-section from Transect II (Figure 2.26) supported the interpretation of a surface water layer moving in an offshore direction. The 36 ppt isohaline defined a lens of somewhat lower salinity that was present in the upper 15 m between Stations 5/II and 3/II.

August Monthly Cruise (August 4, 1977)

The temperature cross-section from Transect II (Figure 2.27) was similar to that constructed from the July data, with warmest surface temperatures of over 29°C found outside Station 4/II and an onshore encroachment of near-bottom water suggested by the 22, 24 and 26° isotherms. The seasonal thermocline over the middle and outer shelf was centered in the layer between approximately 40 and 50 meters.

Top to near-bottom salinity values were 36.25 ± 0.15 ppt (Figure 2.28). No spatial continuity was apparent, either horizontally or vertically, within this range, and thus isohalines were not entered.

Fall Seasonal Cruise Data

Transect I (September 10-11, 1977)

Temperatures along Transect I were well mixed through the upper 20-30 m (Figure 2.29). A very well defined seasonal thermocline, capped by the 29° isotherm, appeared just above the bottom at Station 2/I. Isotherms diverged rapidly with increasing distance from the coast. The greatest vertical temperature gradients were at Station 3/I centered at approximately 50 m. Sur-

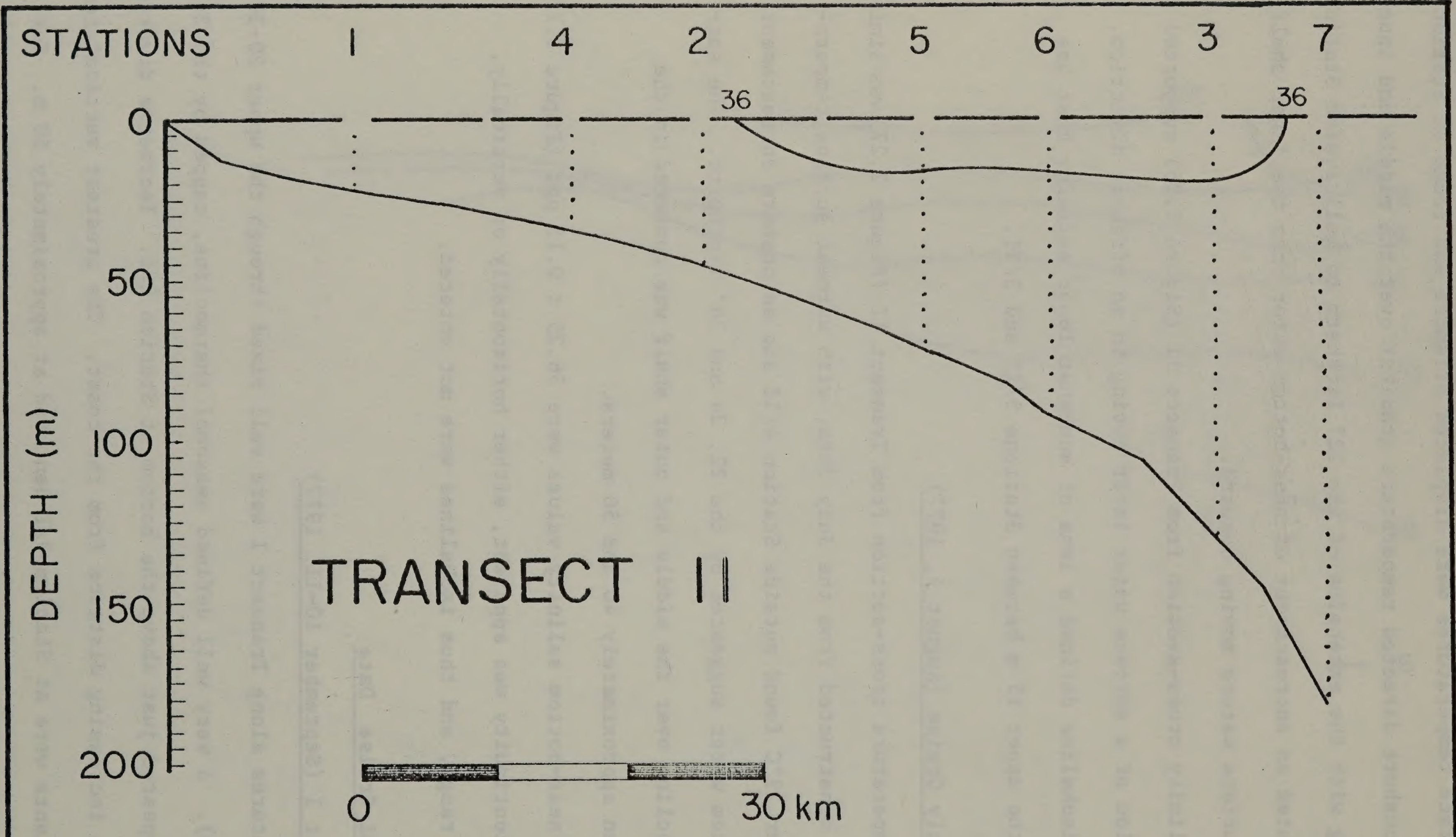

Figure 2.26 July Salinities (ppt) for 6 July 1977.

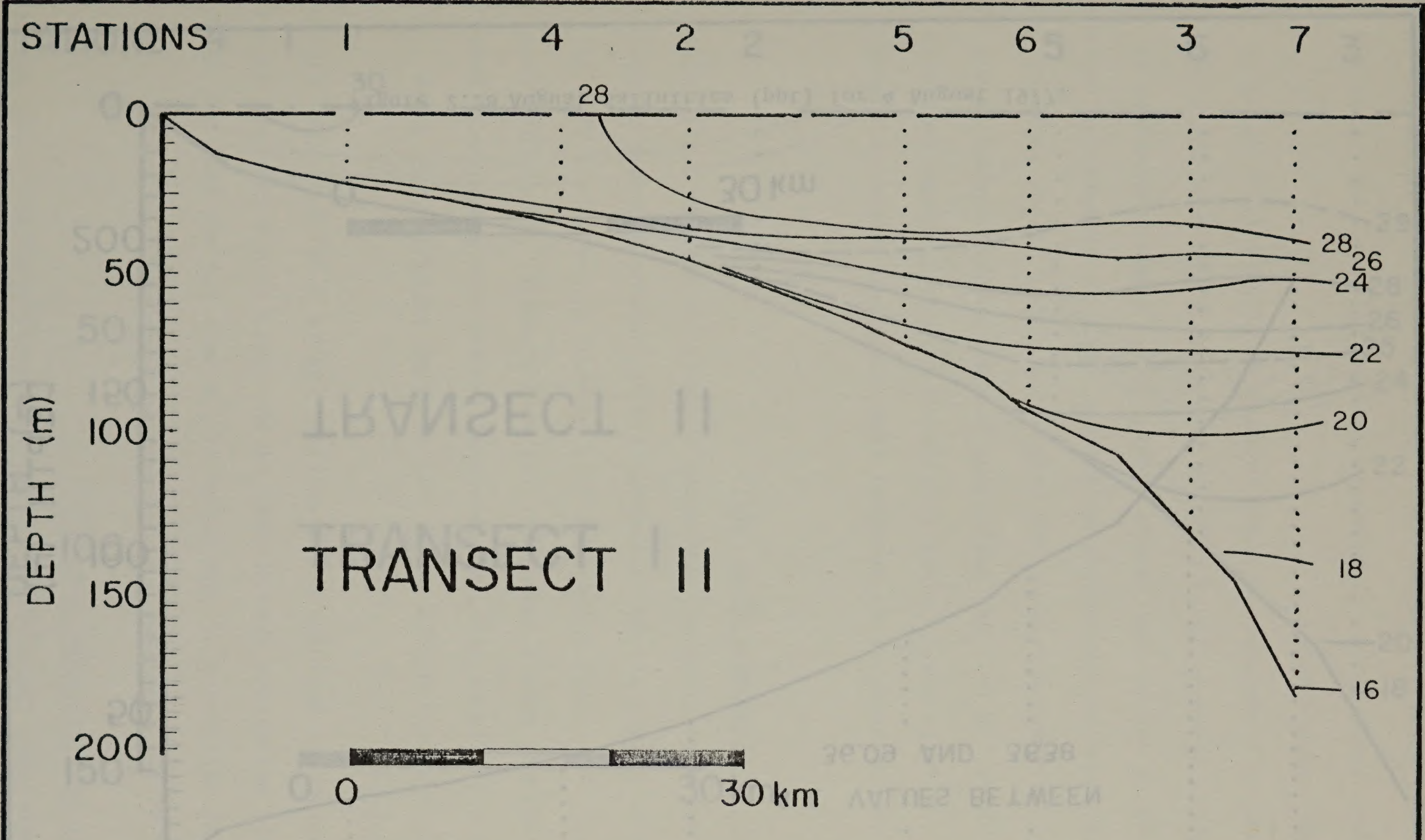

Figure 2.27 August Temperatures (°C) for 4 August 1977.

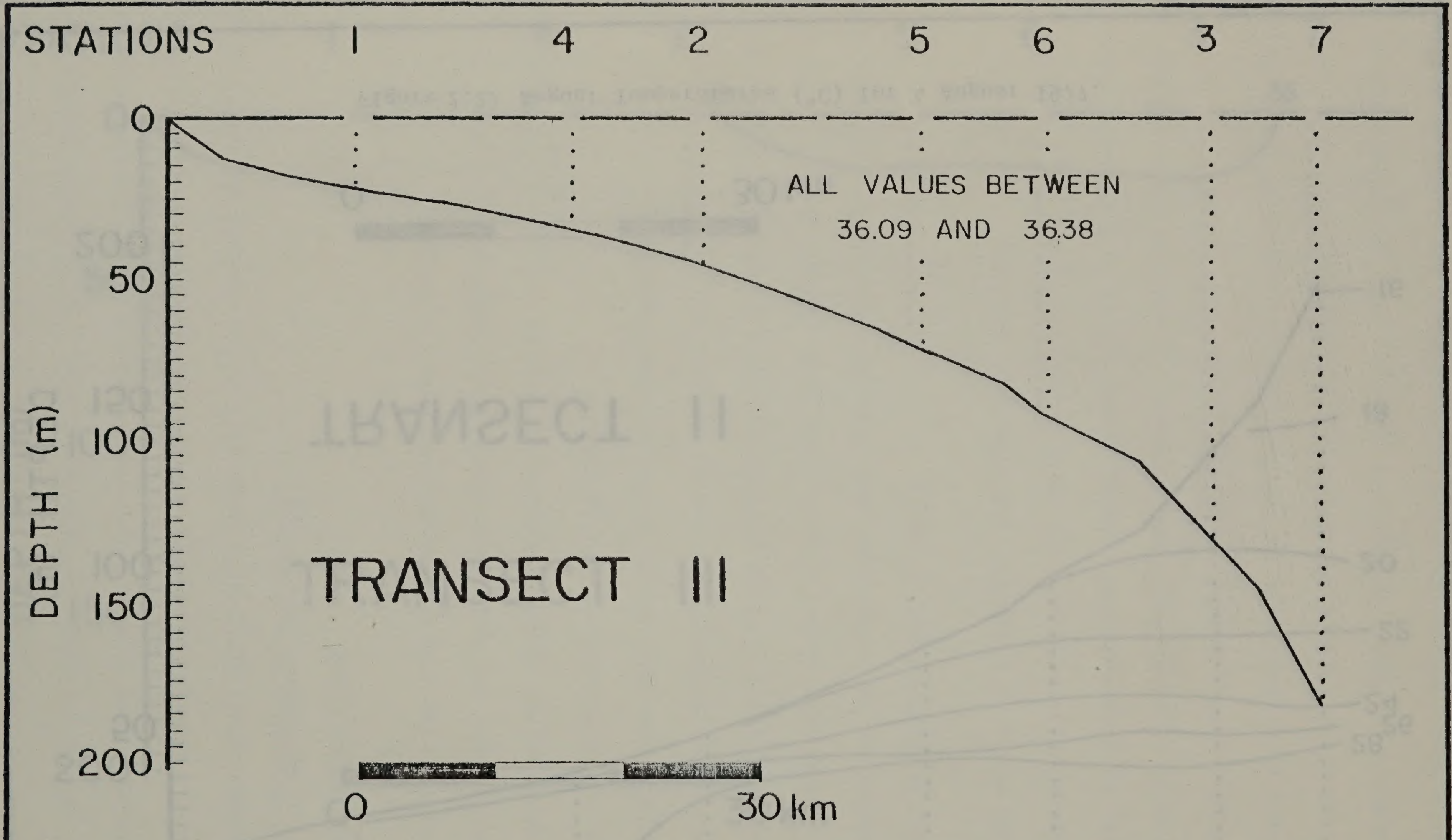

Figure 2.28 August Salinities (ppt) for 4 August 1977.

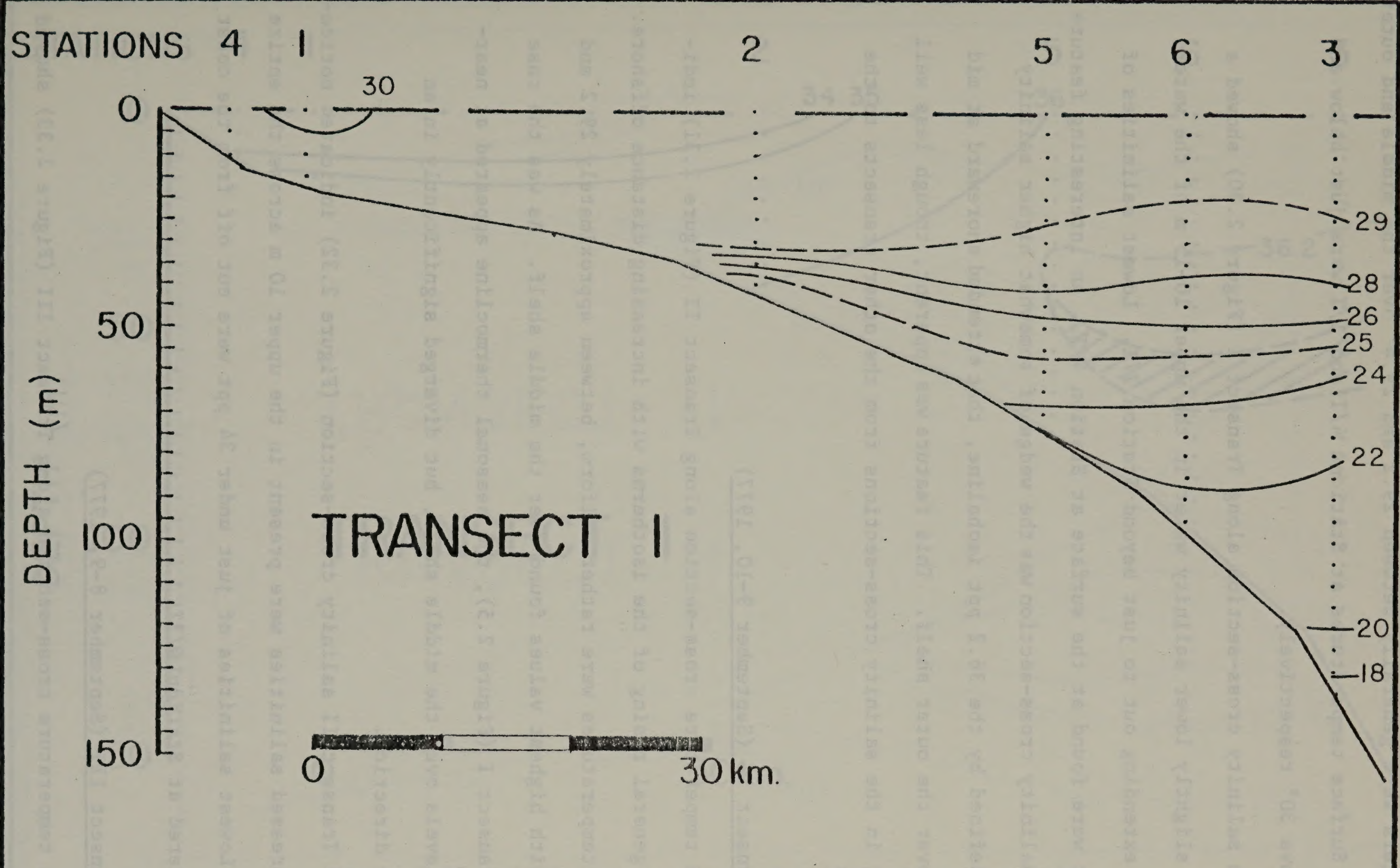

Figure 2.29 Fall Temperatures (°C) for 10-11 September 1977.

face waters were generally between 29.2 and 29.4° over the middle and outer shelf. Surface temperatures at Stations 4/I and 1/I were just below and just above 30° respectively.

The salinity cross-section along Transect I (Figure 2.30) showed a lens of slightly lower salinity water in the upper 10-15 m of the water column, extending out to just beyond Station 2/I. Lowest salinities of 30.0 ppt were found at the surface at Station 4/I. An interesting feature of the salinity cross-section was the wedge of somewhat higher salinity water, defined by the 36.2 ppt isohaline, that extended shoreward at mid depths over the outer shelf. This feature was apparent, though less well defined, in the salinity cross-sections from the other transects to the south.

Transect II (September 9-10, 1977)

The temperature cross-section along Transect II (Figure 2.31) indicated a general rising of the isotherms with increasing distance offshore. Surface temperatures were rather uniform, between approximately 29.2 and 29.8°, with highest values found over the middle shelf. As was the case along Transect I (Figure 2.5), the seasonal thermocline appeared at near-bottom levels over the middle shelf, but diverged significantly in an offshore direction.

The Transect II salinity cross-section (Figure 2.32) indicated noticeably depressed salinities were present in the upper 10 m across the entire shelf. Lowest salinities of just under 34 ppt were cut off from the coast and centered at Station 4/II.

Transect III (September 8-9, 1977)

The temperature cross-section along Transect III (Figure 2.33) showed a substantially thicker layer of the water column was present above the

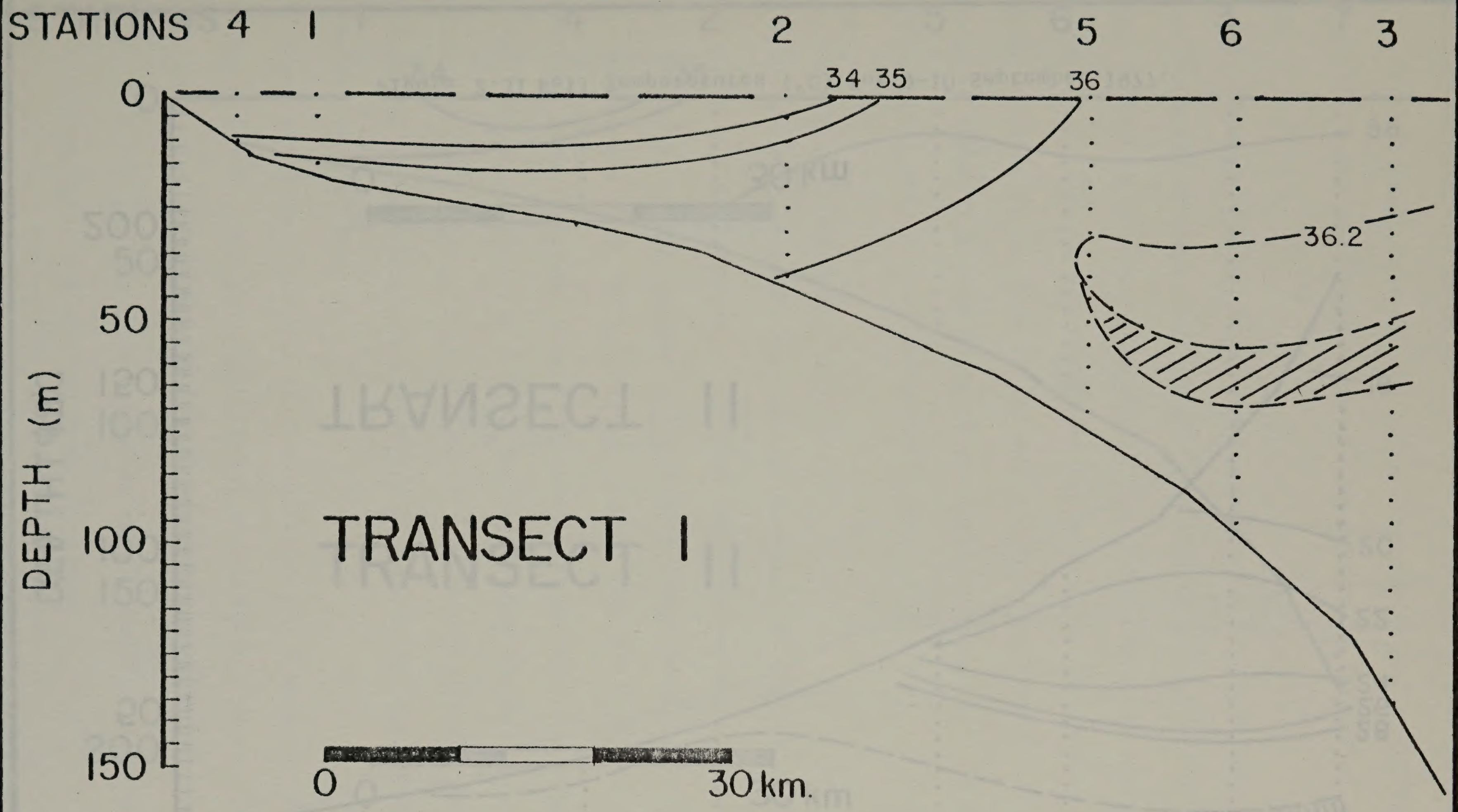

Figure 2.30 Fall Salinities (ppt) for 10-11 September 1977.

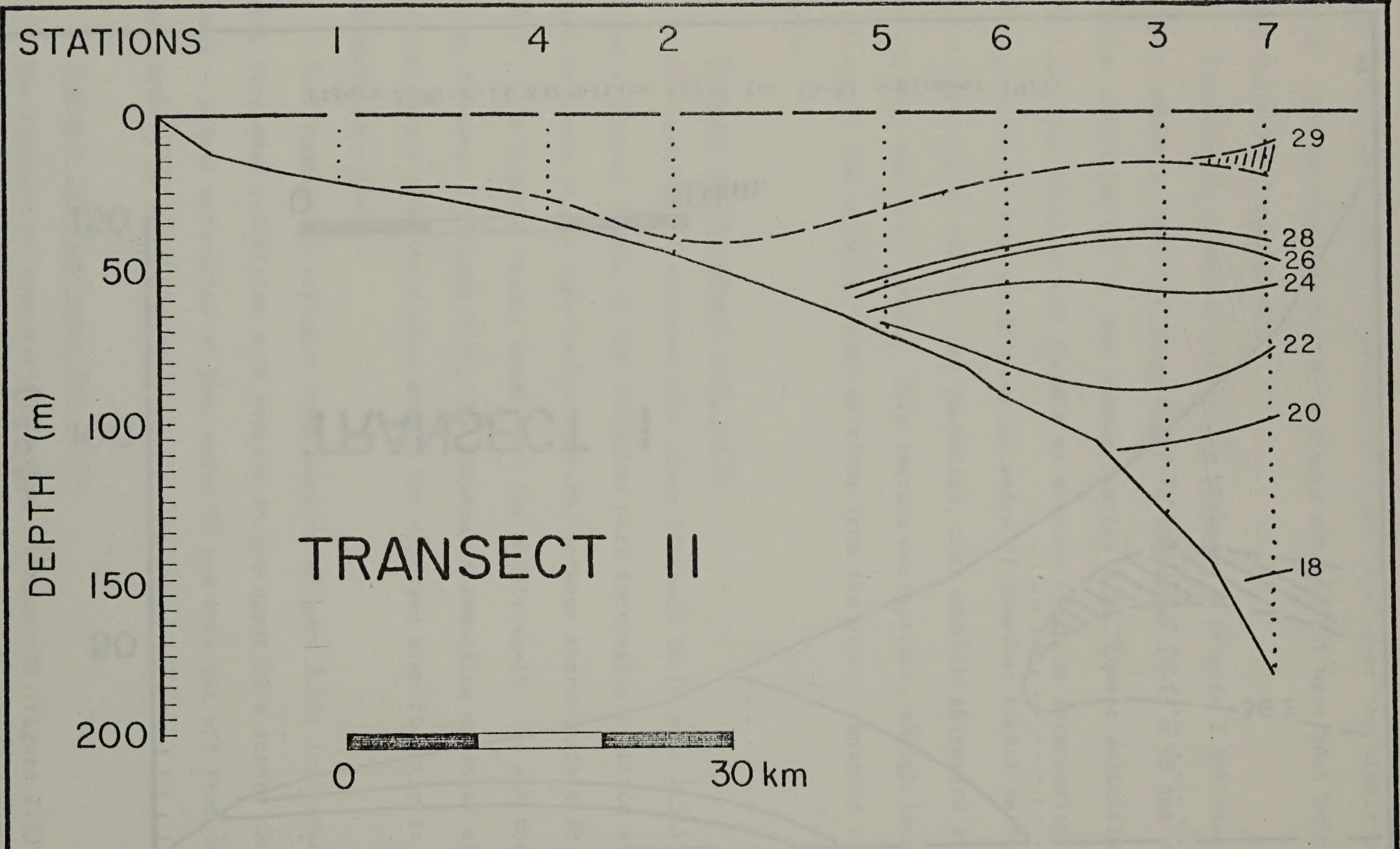

Figure 2.31 Fall Temperatures (°C) for 9-10 September 1977.

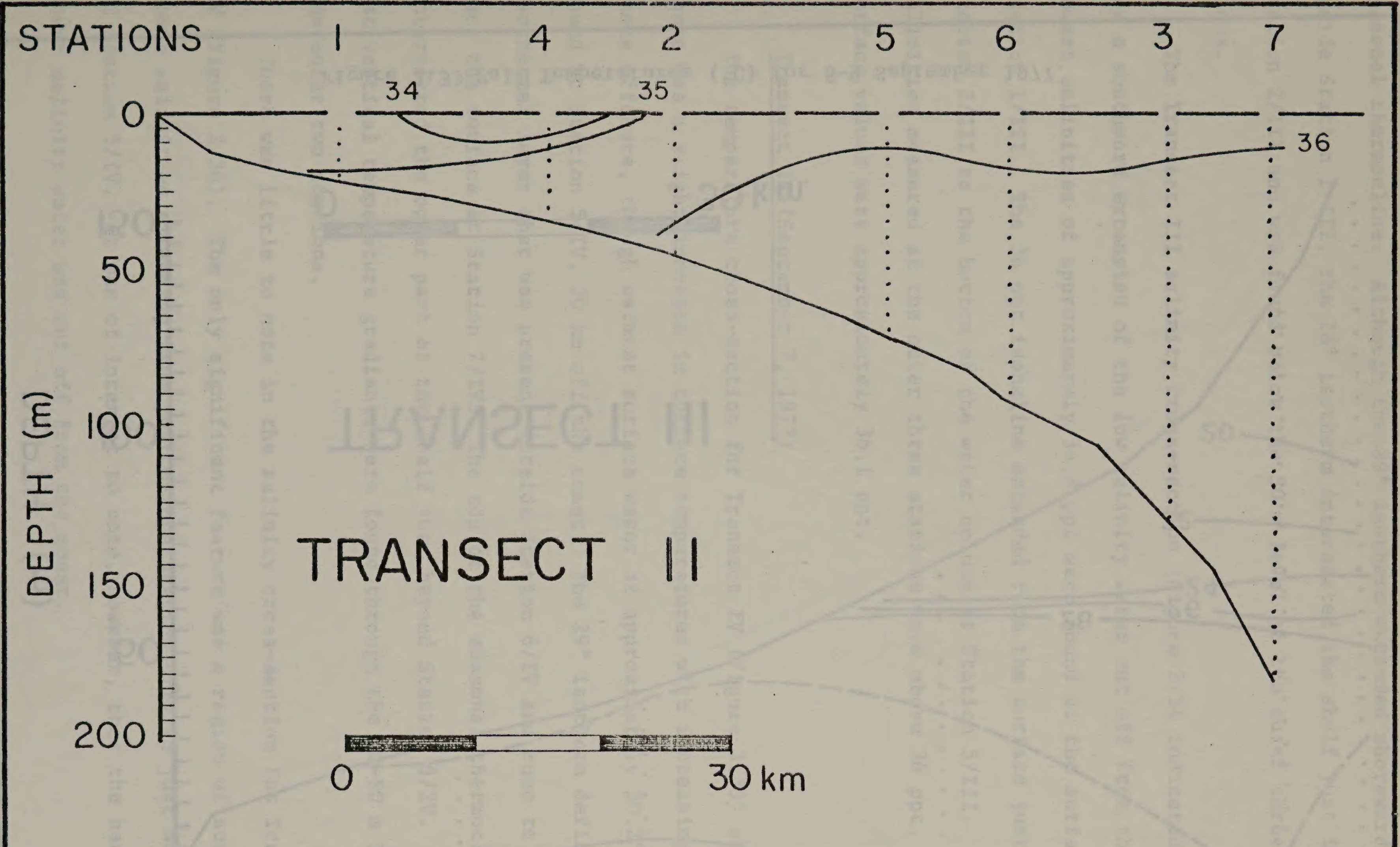

Figure 2.32 Fall Salinities (ppt) for 9-10 September 1977.

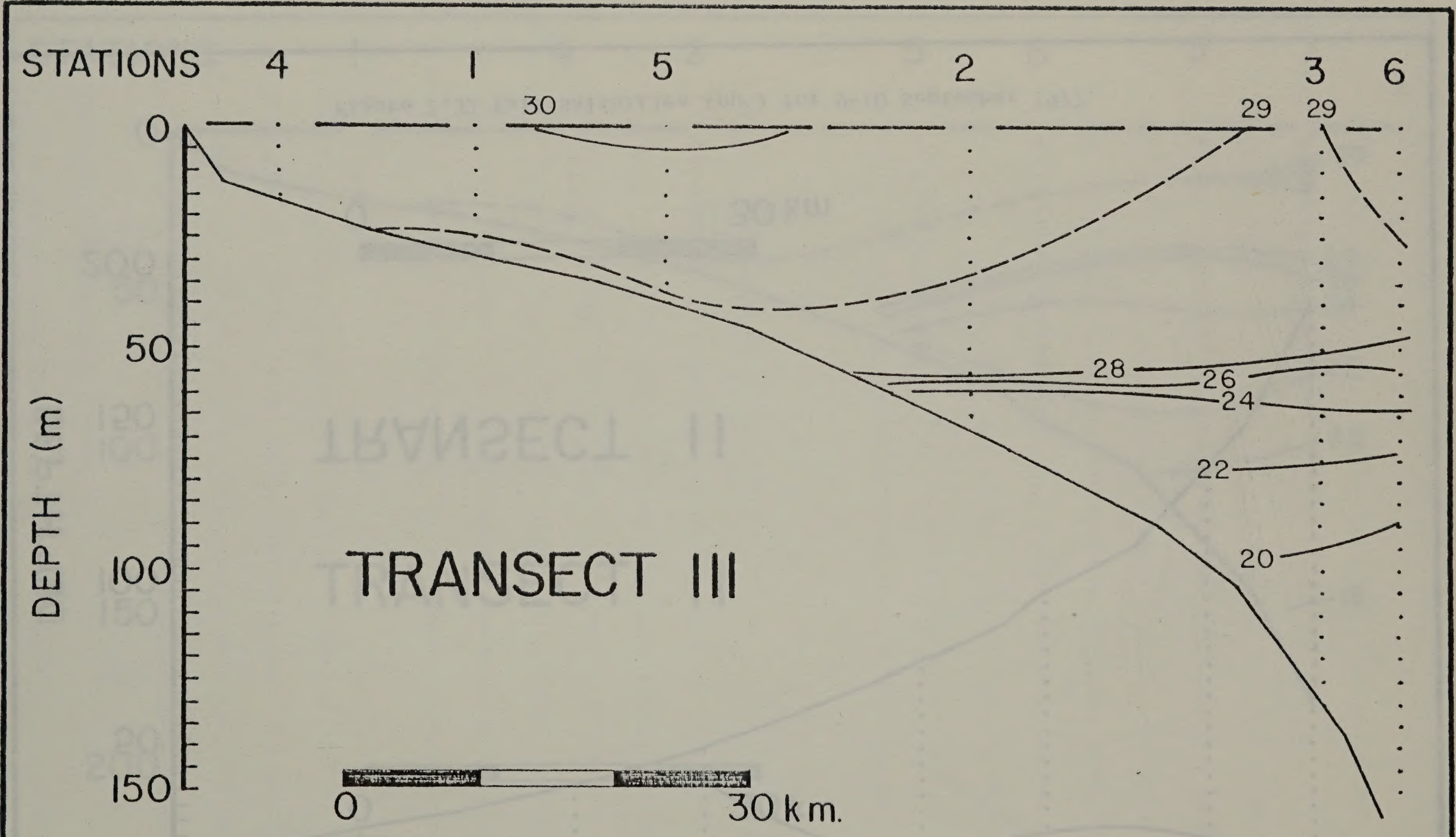

Figure 2.33 Fall Temperatures (°C) for 8-9 September 1977.

seasonal thermocline. Although the 29° isotherm extended shoreward to inside Station 1/III, the 28° isotherm intersected the shelf just inside Station 2/III and was found below the 50 m level at the outer three stations.

The Transect III salinity cross-section (Figure 2.34 indicated there was a southward extension of the low salinity water cut off from the coast. Lowest salinities of approximately 34.8 ppt were found at the surface at Station 1/III. The 36 ppt isohaline extended from the surface just inside Station 2/III to the bottom of the water column at Station 5/III. All salinities measured at the outer three stations were above 36 ppt, though surface values were approximately 36.1 ppt.

Transect IV (September 7, 1977)

The temperature cross-section for Transect IV (Figure 2.35) showed there was a slight increase in surface temperatures with increasing distance offshore, though warmest surface water of approximately 30.2° was found at Station 5/IV, 30 km off the coast. The 29° isotherm defined an isothermal layer that was present outside Station 6/IV and rose to intersect the surface at Station 7/IV. The top of the seasonal thermocline intersected the outer part of the shelf just beyond Station 6/IV. Strongest vertical temperature gradients were found through the 50-80 m layer at the outer two stations.

There was little to note in the salinity cross-section for Transect IV (Figure 2.36). The only significant feature was a region of somewhat lower salinities extending from just beyond Station 4/IV to just shoreward of Station 5/IV. It was of interest to note, however, that the band of lower salinity water was cut off from the coast.

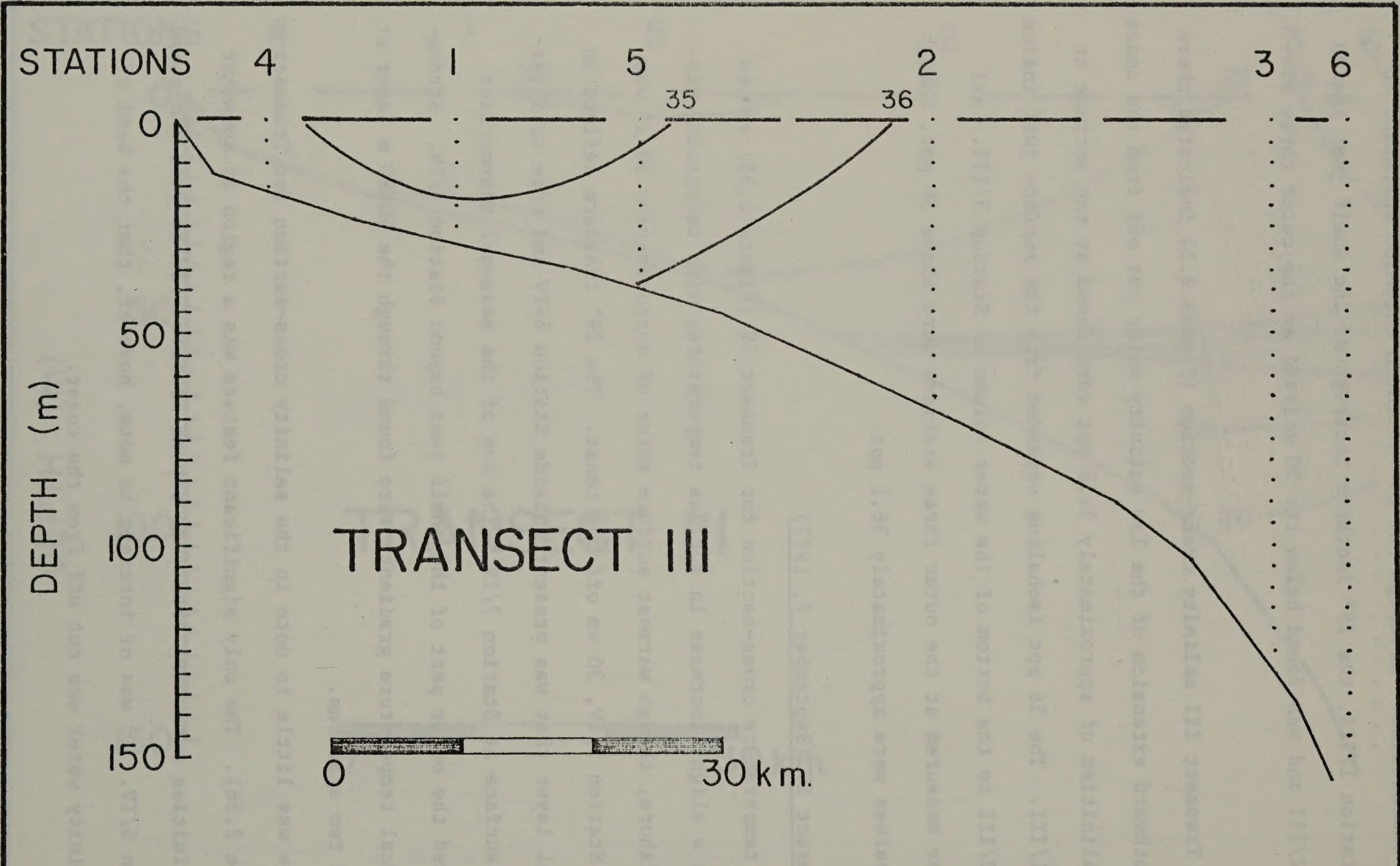

Figure 2.34 Fall Salinities (ppt) for 8-9 September 1977.

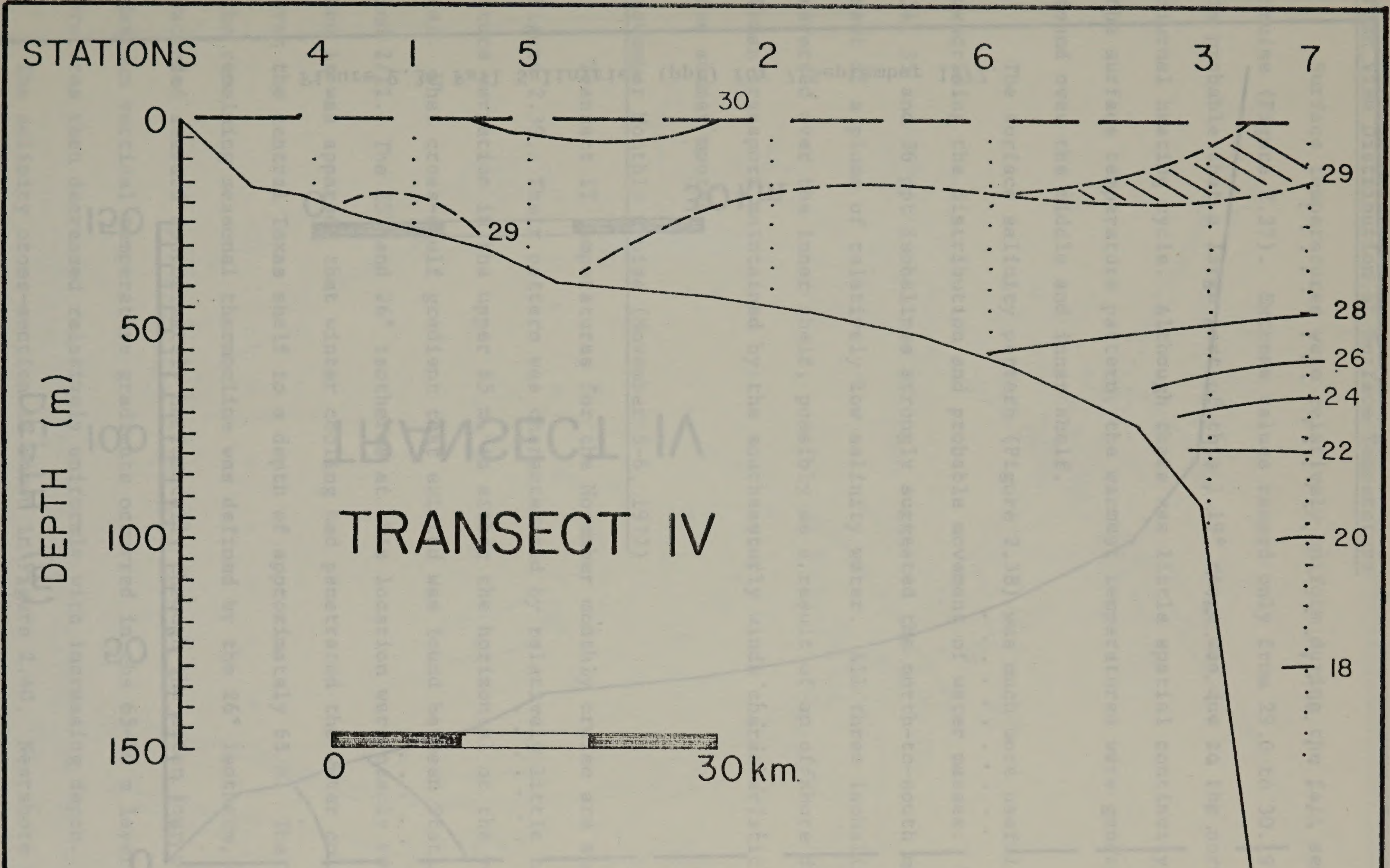

Figure 2.35 Fall Temperatures (°C) for 7 September 1977.

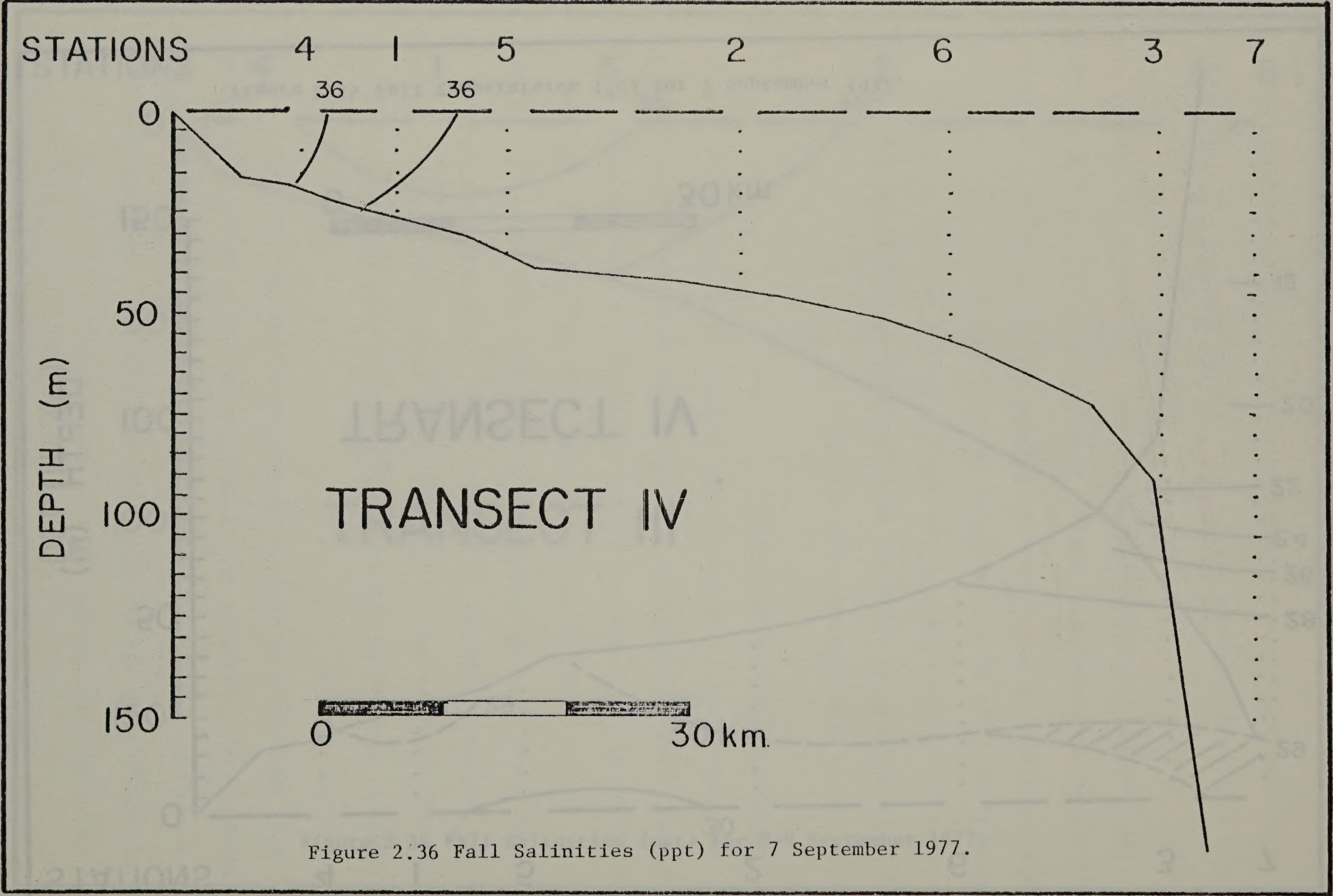

Figure 2.36 Fall Salinities (ppt) for 7 September 1977.

Plan View Distribution of Surface Temperature

Surface temperatures were relatively uniform during the fall seasonal cruise (Figure 2.37). Extreme values ranged only from 29.0 to 30.19°C. It is probable that a large part of this 1.19° range was due to the normal diurnal heating cycle. Although there was little spatial continuity in the surface temperature pattern, the warmest temperatures were generally found over the middle and inner shelf.

The surface salinity pattern (Figure 2.38) was much more useful in describing the distribution and probable movement of water masses. The 34, 35 and 36 ppt isohalines strongly suggested the north-to-south movement of a plume of relatively low salinity water. All three isohalines reversed over the inner shelf, possibly as a result of an offshore directed Ekman transport maintained by the southeasterly winds characteristic of the summer months.

November Monthly Cruise (November 5-6, 1977)

Transect II temperatures for the November monthly cruise are shown in Figure 2.39. Their pattern was characterized by relatively little temperature variation in the upper 65 m, in either the horizontal or the vertical. What cross-shelf gradient that existed was found between Station 4/II and 2/II. The 25° and 26° isotherms at this location were nearly vertical, and it was apparent that winter cooling had penetrated the water column over the central Texas shelf to a depth of approximately 65 m. The top of the remaining seasonal thermocline was defined by the 26° isotherm, which extended seaward approximately horizontally through the outer four profiles. Maximum vertical temperature gradients occurred in the 65-80 m layer; temperatures then decreased relatively uniformly with increasing depth.

The salinity cross-section is shown in Figure 2.40. Nearshore water was only slightly diluted by continental runoff, and all salinities were

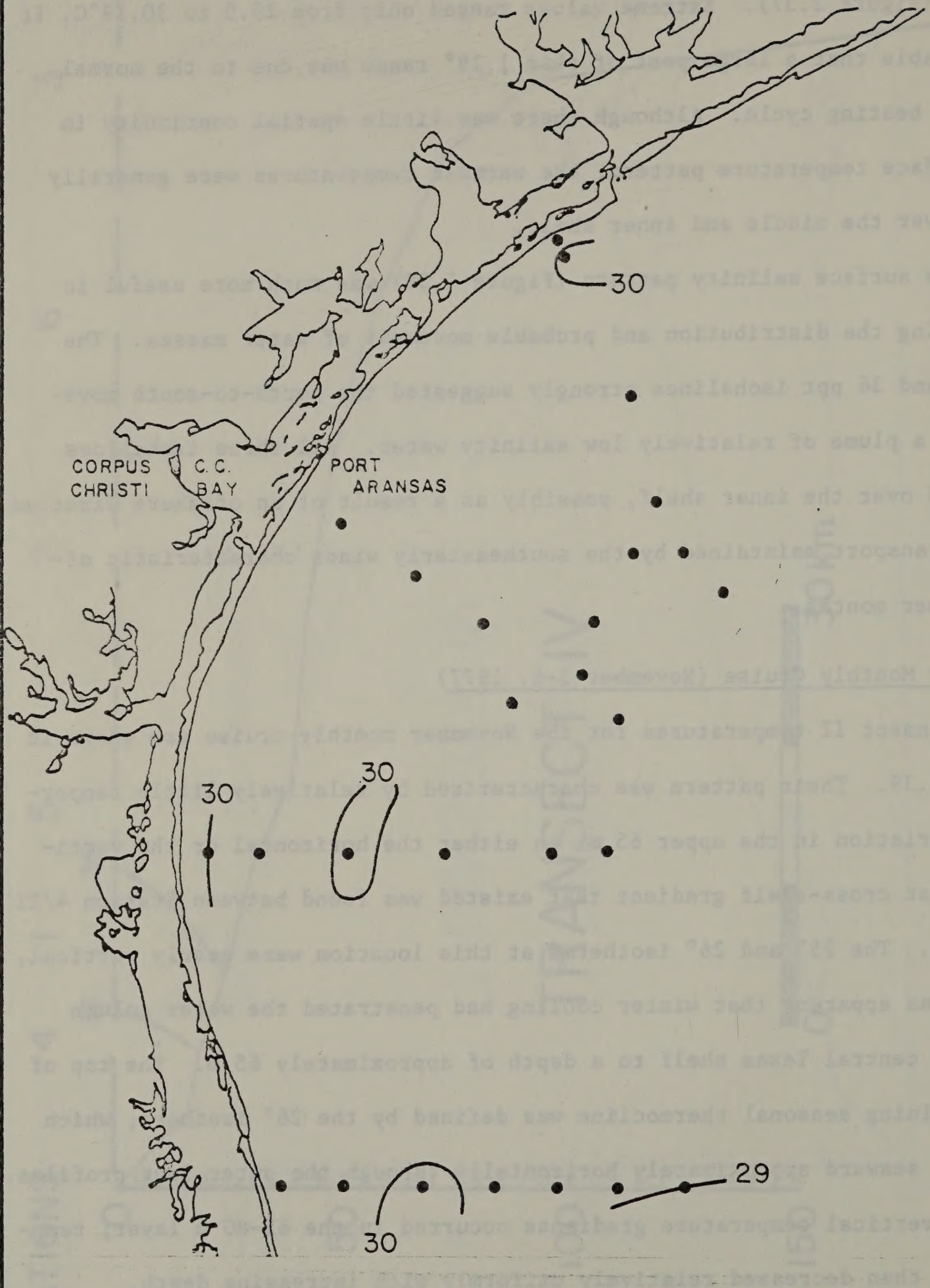

Figure 2.37 Fall Surface Temperature (°C) Pattern, 7-11 September 1977.

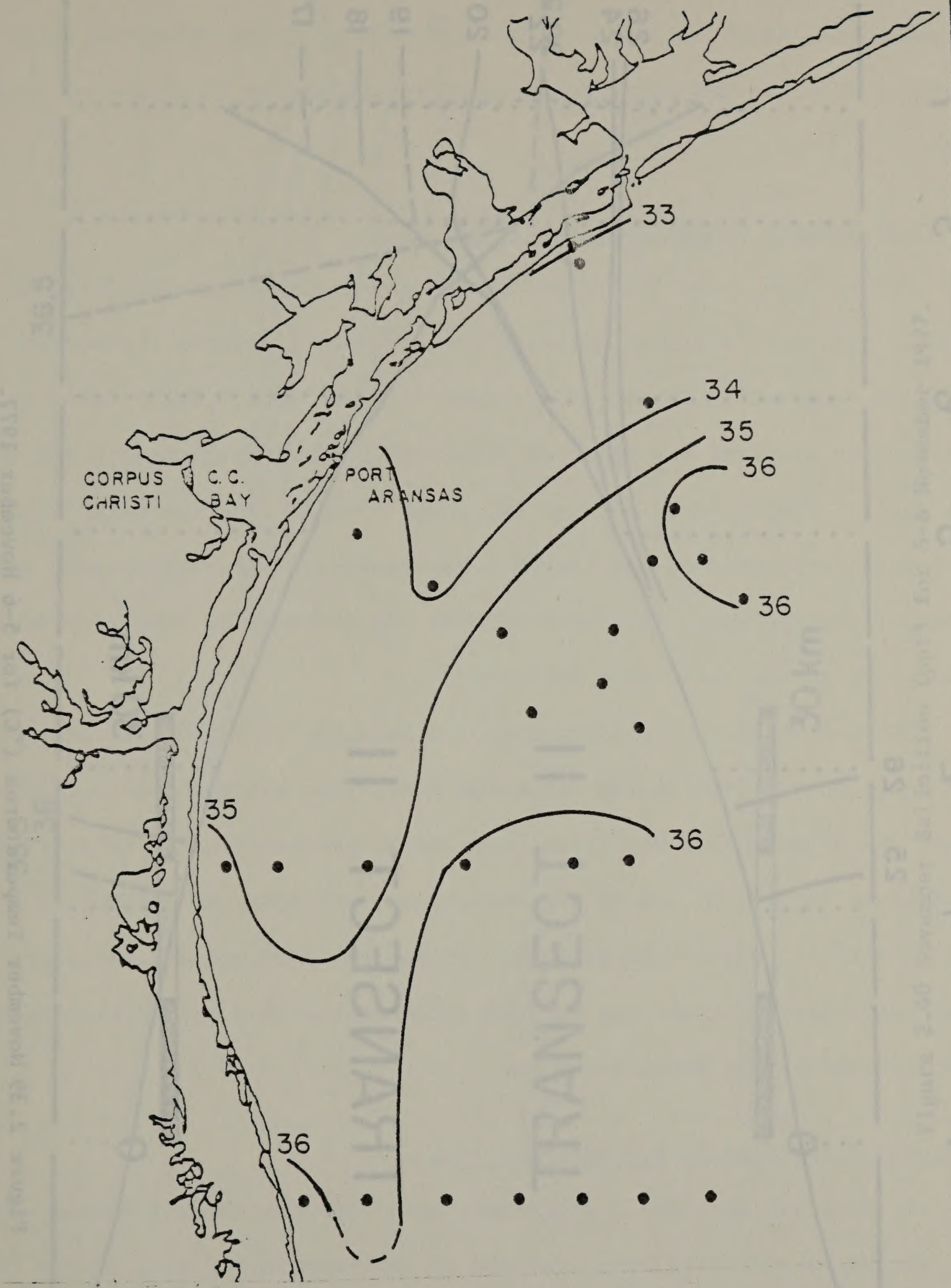

Figure 2.38 Fall Surface Salinity (ppt) Pattern, 7-11 September 1977.

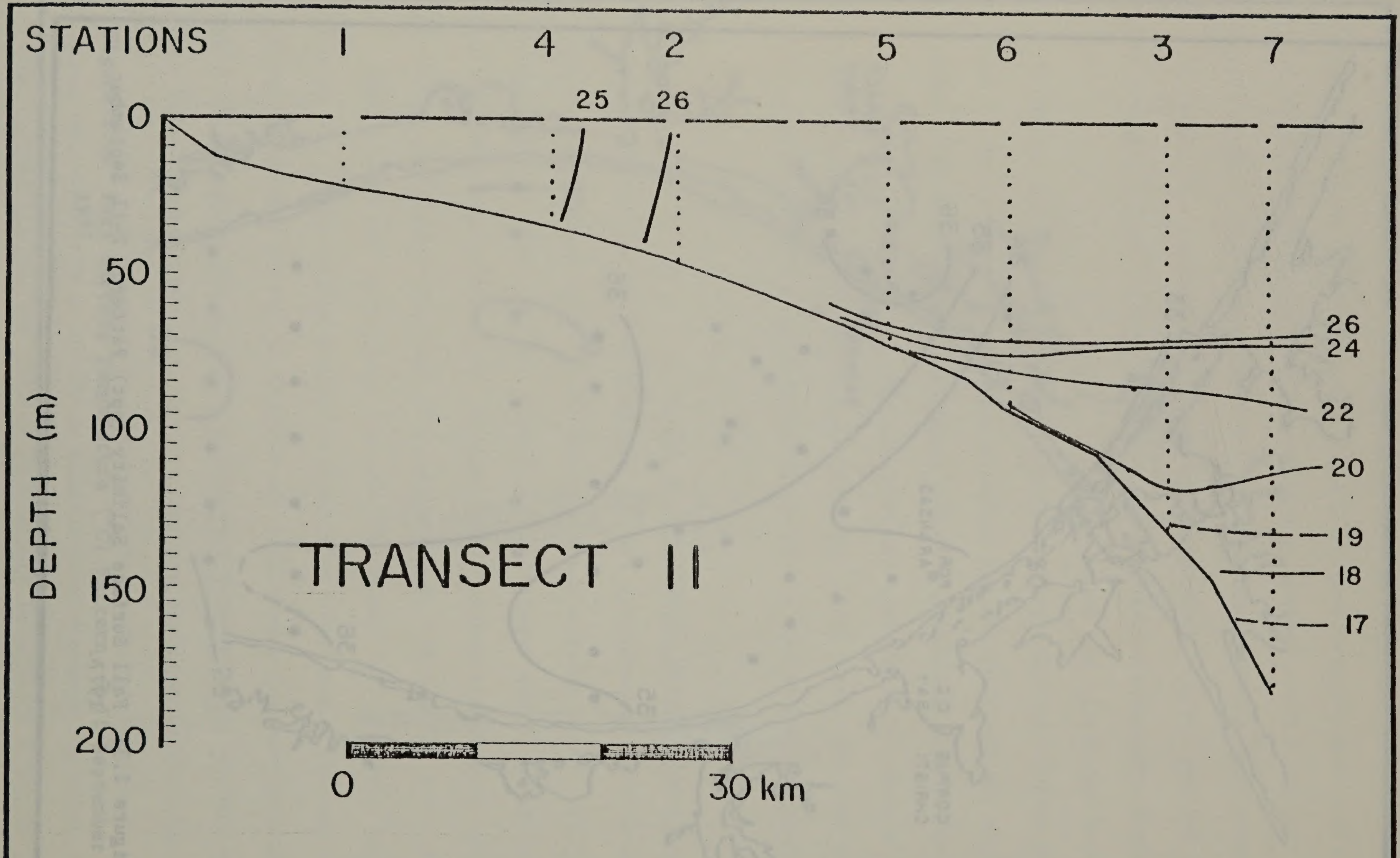

Figure 2.39 November Temperatures (°C) for 5-6 November 1977.

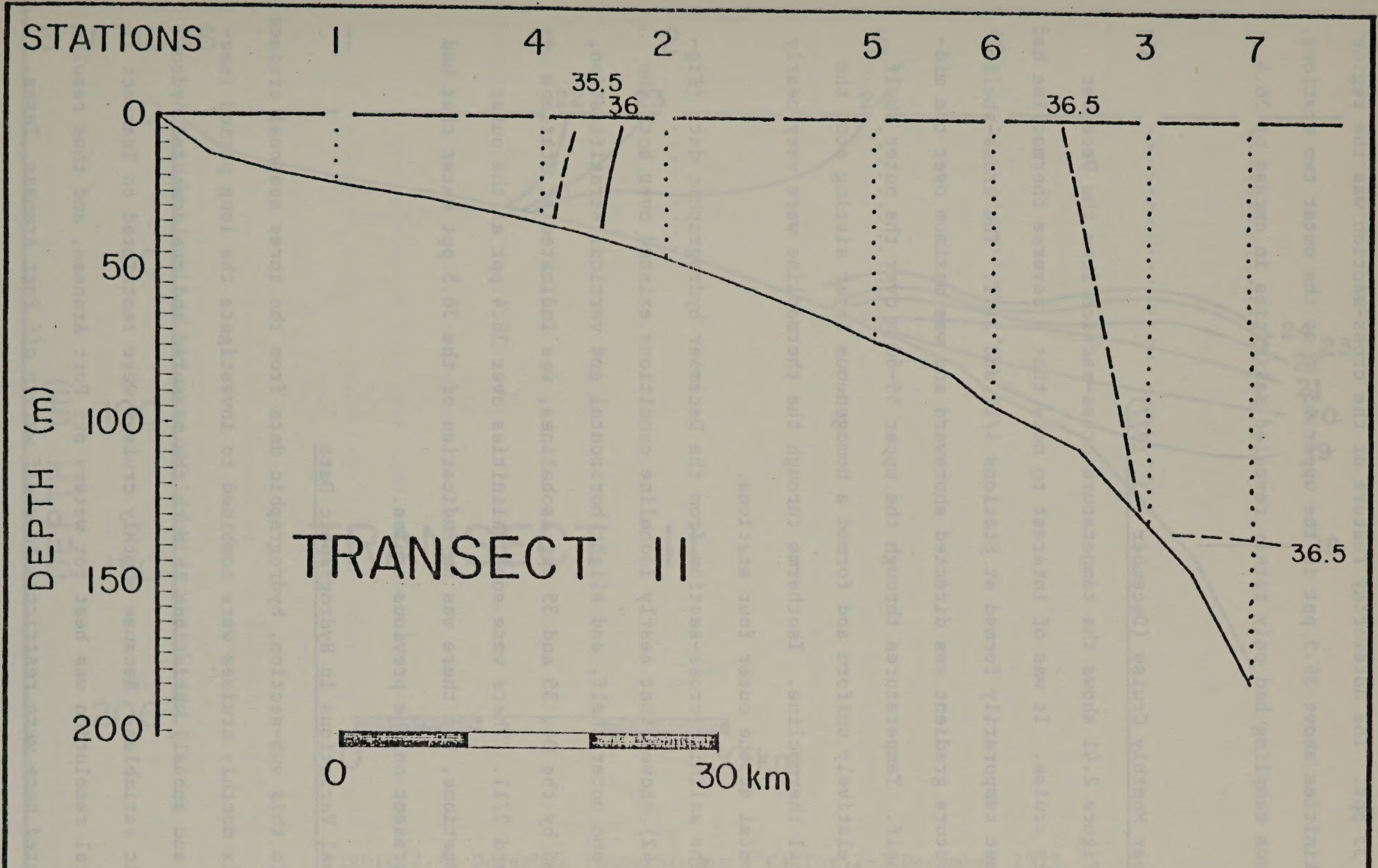

Figure 2.40 November Salinities (ppt) for 5-6 November 1977.

above 35 ppt. The noteworthy feature of the cross-section was the region of salinities above 36.5 ppt in the upper 130 m at the outer two stations. Previous sampling had only rarely revealed salinities in excess of 36.4 ppt.

December Monthly Cruise (December 2-3, 1977)

Figure 2.41 shows the temperature cross-section from the December monthly cruise. It was of interest to note that a reverse thermocline had at least temporarily formed at Stations 4/II and 2/II. The cross-shelf temperature gradient was directed shoreward and was maximum over the middle shelf. Temperatures through the upper 55-60 m over the outer shelf were relatively uniform and formed a homogeneous layer sitting atop the seasonal thermocline. Isotherms through the thermocline were very nearly horizontal at the outer four stations.

The salinity cross-section from the December hydrographic data (Figure 2.42) showed that nearly isohaline conditions existed over both the inner and outer shelf, and slight horizontal and vertical stratification, defined by the 34, 35 and 35 ppt isohalines, was indicated at Stations 4/II and 2/II. There were some salinities over 36.4 ppt at the outer four stations, but there was no indication of the 36.5 ppt water that had been present on the previous cruise.

Seasonal Variations in Hydrographic Data

In this sub-section, hydrographic data from the three seasonal cruises and six monthly cruises were combined to investigate the long period (seasonal and annual) variations in both the measured and the computed hydrographic variables. Because monthly cruises were restricted to Transect II temporal resolution was best for waters off Port Aransas, and thus results presented here were restricted to shelf waters off Port Aransas, Texas.

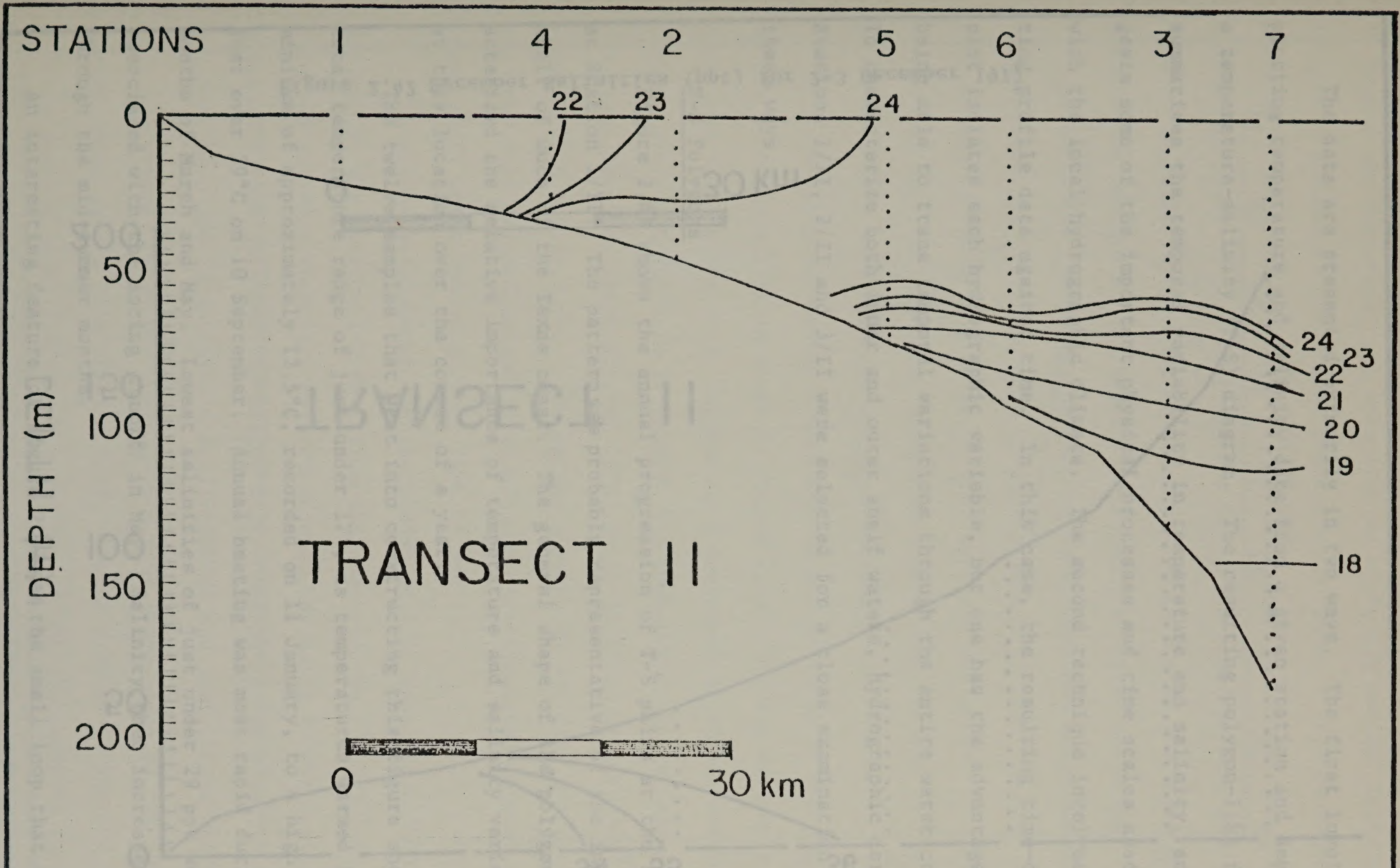

Figure 2.41 December Temperatures (°C) for 2-3 December 1977.

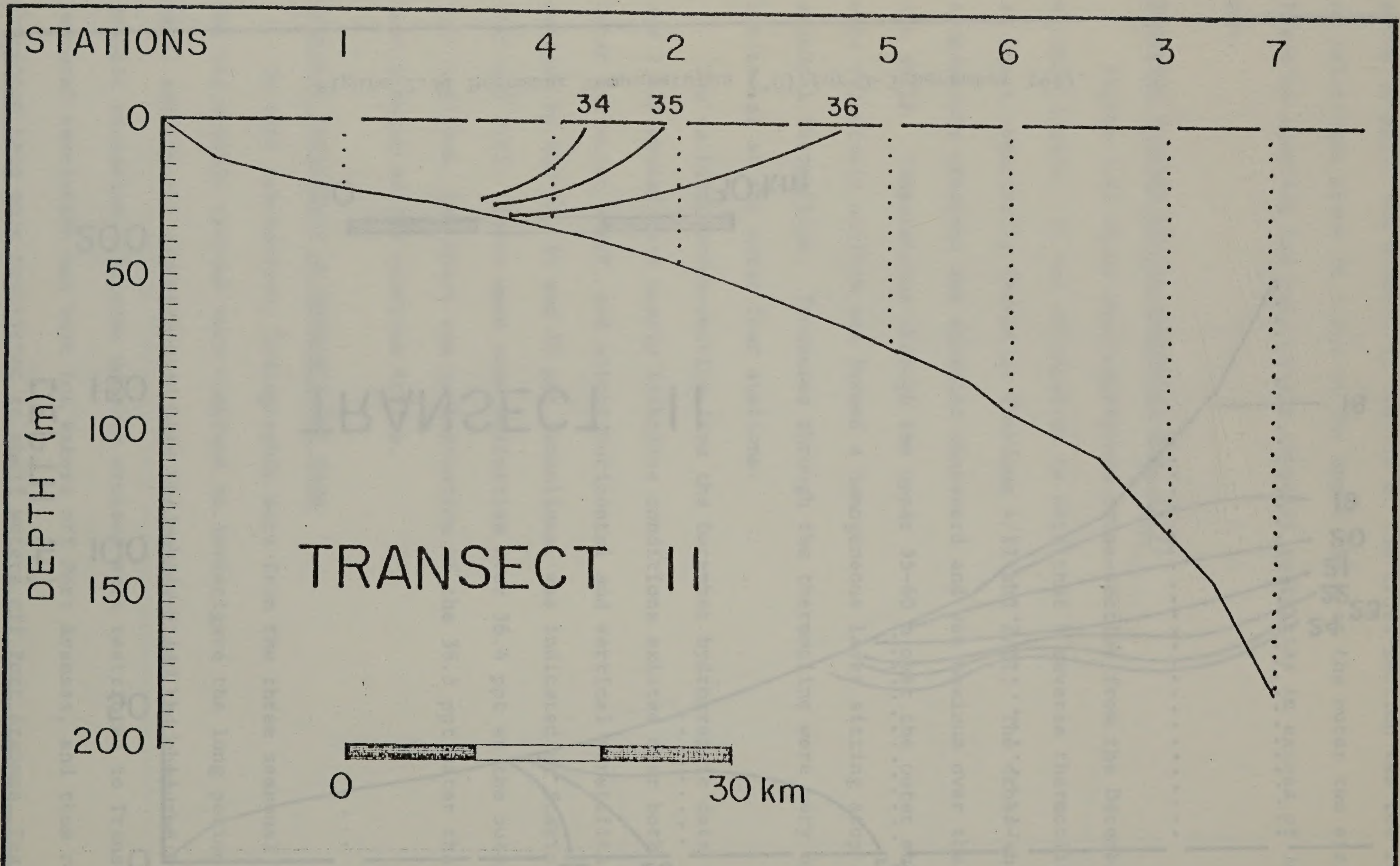

Figure 2.42 December Salinities (ppt) for 2-3 December 1977.

The data are presented primarily in two ways. The first involves plotting temperature and salinity data from a given station and depth on a temperature-salinity (T-S) diagram. The resulting polygon-like figure summarizes the temporal variability in temperature and salinity, and suggests some of the important physical processes and time scales associated with the local hydrographic climate. The second technique involves plotting profile data against time. In this case, the resulting time-depth plot isolates each hydrographic variable, but one has the advantage of being able to trace temporal variations through the entire water column. To characterize both inner and outer shelf waters, hydrographic data from Stations 1/II, 2/II and 3/II were selected for a close examination in these ways.

T-S Polygons

Figure 2.43 shows the annual progression of T-S pairs at the surface at Station 1/II. The pattern was probably representative of the inner shelf of much of the Texas coast. The general shape of the polygon characterized the relative importance of temperature and salinity variations at this location over the course of a year.

The twelve samples that went into constructing this figure showed a total temperature range of just under 17°, as temperatures warmed from a minimum of approximately 12.5°C, recorded on 11 January, to a high of just over 29°C on 10 September. Annual heating was most rapid during the months of March and May. Lowest salinities of just under 29 ppt were associated with the spring runoff in May. Salinity then increased rapidly through the mid-summer months.

An interesting feature of Figure 2.43 was the small loop that occurred

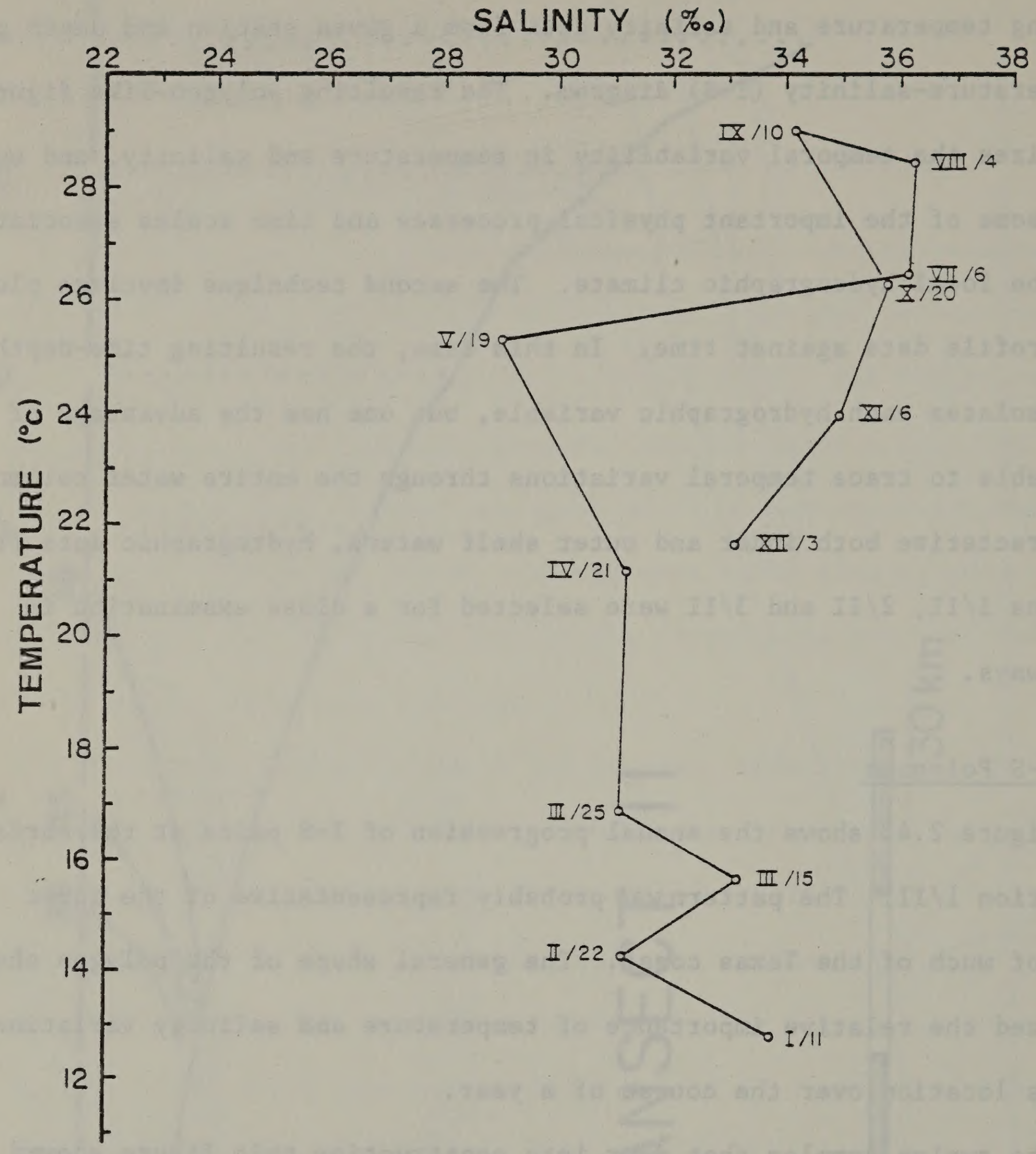

Figure 2.43 Annual Progression of Temperature-Salinity Pairs at the Surface of Station 1/II.

in August and September. At this time of year, the central Texas coast records a maximum in the annual precipitation curve. This decreases the salinity of shelf waters slightly, and with only minor additional heating before the onset of fall cooling, the T-S pairs trace out the observed loop. This same feature was recorded in the 1976 data and appeared to be a recurring feature in the hydrographic cycle of these inner shelf waters.

Although the 1977 sampling program continued into early December, temperatures had decreased only to approximately 22°C by the last cruise. This suggested that the greater part of the fall and winter cooling began around the first of the year, when cold fronts that arrived at the Texas coast were both more frequent and more intense.

The annual progression of temperature and salinity at near-bottom levels at Station 1/II is shown in Figure 2.44. The pattern was elongated along the temperature axis, suggesting a dominance of annual heating and cooling over variations in salinity. The temperature range indicated by the available data was just over 18°C, while salinity measurements were between 31.8 and 36.3 ppt. The spring minimum in salinity was apparent but less well defined and indicated salinity stratification through the inner shelf water column at that time of year.

The mid to late summer counter-clockwise loop was larger than that defined by the surface T-S data from the same station (Figure 2.43). This was due to a temporary interruption in the early summer heating revealed by the July Monthly Cruise data. This brief near-bottom cooling may have been due to an onshore deflection of the near-bottom current.

T-S variations in mid-shelf waters were illustrated by surface and bottom measurements from Station 2/II. Figure 2.45 gives the T-S polygon for the surface layer. With the exception of the low salinity recorded on 19 May, the pattern was characterized by a quite narrow range of salinity

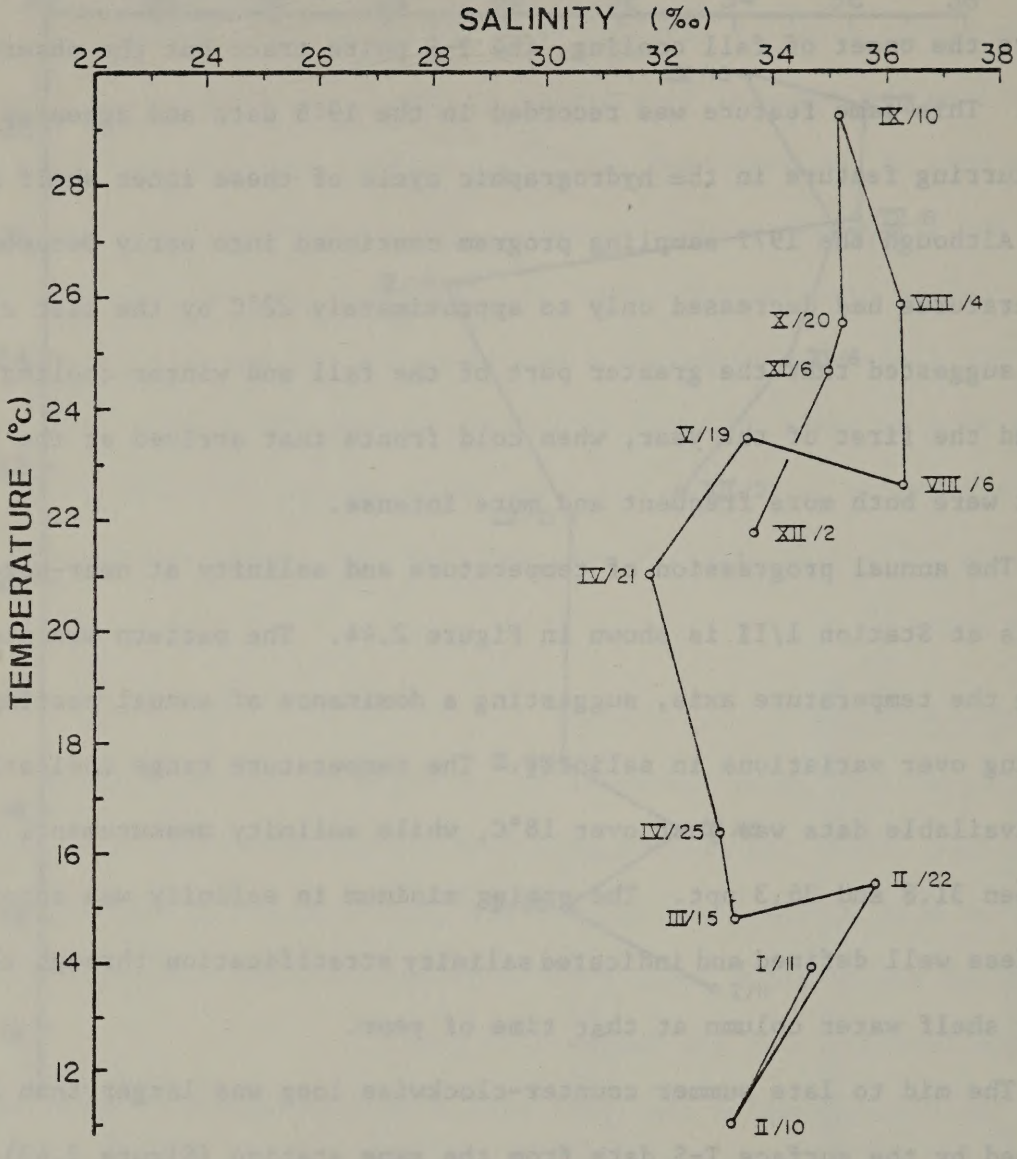

Figure 2.44 Annual Progression of Temperature-Salinity Pairs at the Bottom of Station 1/II.

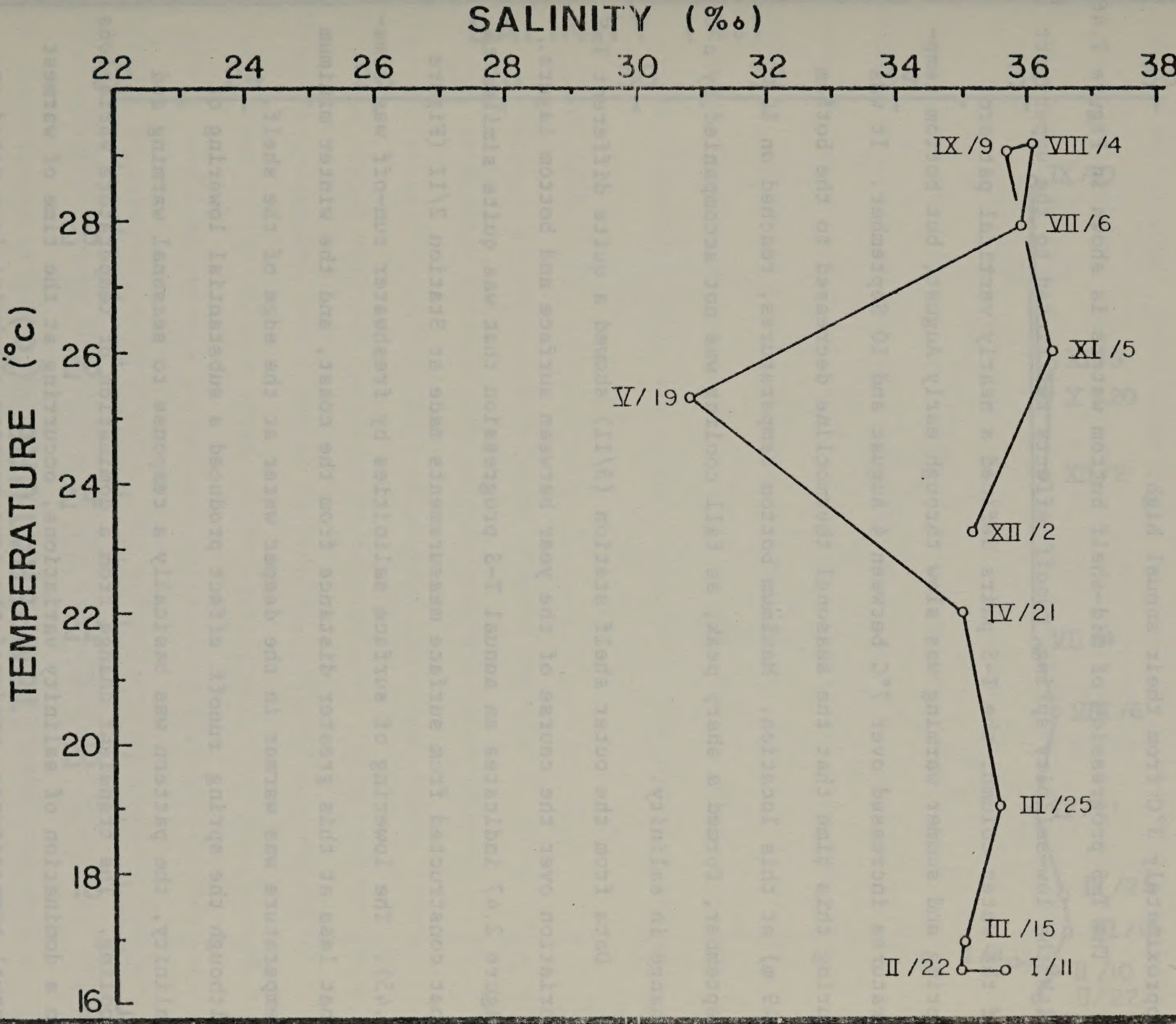

Figure 2.45 Annual Progression of Temperature-Salinity Pairs at the Surface of Station 2/II.

and a dominance of annual heating and cooling. Warmest temperatures of just over 29°C in August and September were accompanied by a slight decrease in salinity to produce a small loop. Fall cooling was well underway by the November Monthly Cruise, as surface waters were cooled approximately 3°C from their annual high.

The T-S progression of mid-shelf bottom waters is shown in Figure 2.46. With the low-salinity spring "runoff" effects restricted to the upper part of the water column, the T-S pairs defined a nearly vertical pattern. Spring and summer warming was slow through early August, but bottom temperatures increased over 7°C between 4 August and 10 September. It was during this time that the seasonal thermocline decreased to the bottom (49 m) at this location. Maximum bottom temperatures, reached on 10 September, formed a sharp peak, as fall cooling was not accompanied by a change in salinity.

Data from the outer shelf station (3/II) showed a quite different T-S variation over the course of the year between surface and bottom layers. Figure 2.47 indicates an annual T-S progression that was quite similar to that constructed from surface measurements made at Station 2/II (Figure 2.45). The lowering of surface salinities by freshwater run-off was somewhat less at this greater distance from the coast, and the winter minimum temperature was warmer in the deeper water at the edge of the shelf. Although the spring "runoff" effect produced a substantial lowering of salinity, the pattern was basically a response to seasonal warming and cooling. The transient change from a domination of temperature variations to a domination of salinity variations, occurring at the time of warmest annual temperatures, produced the same counter-clockwise loop noted at both stations farther up on the continental shelf.

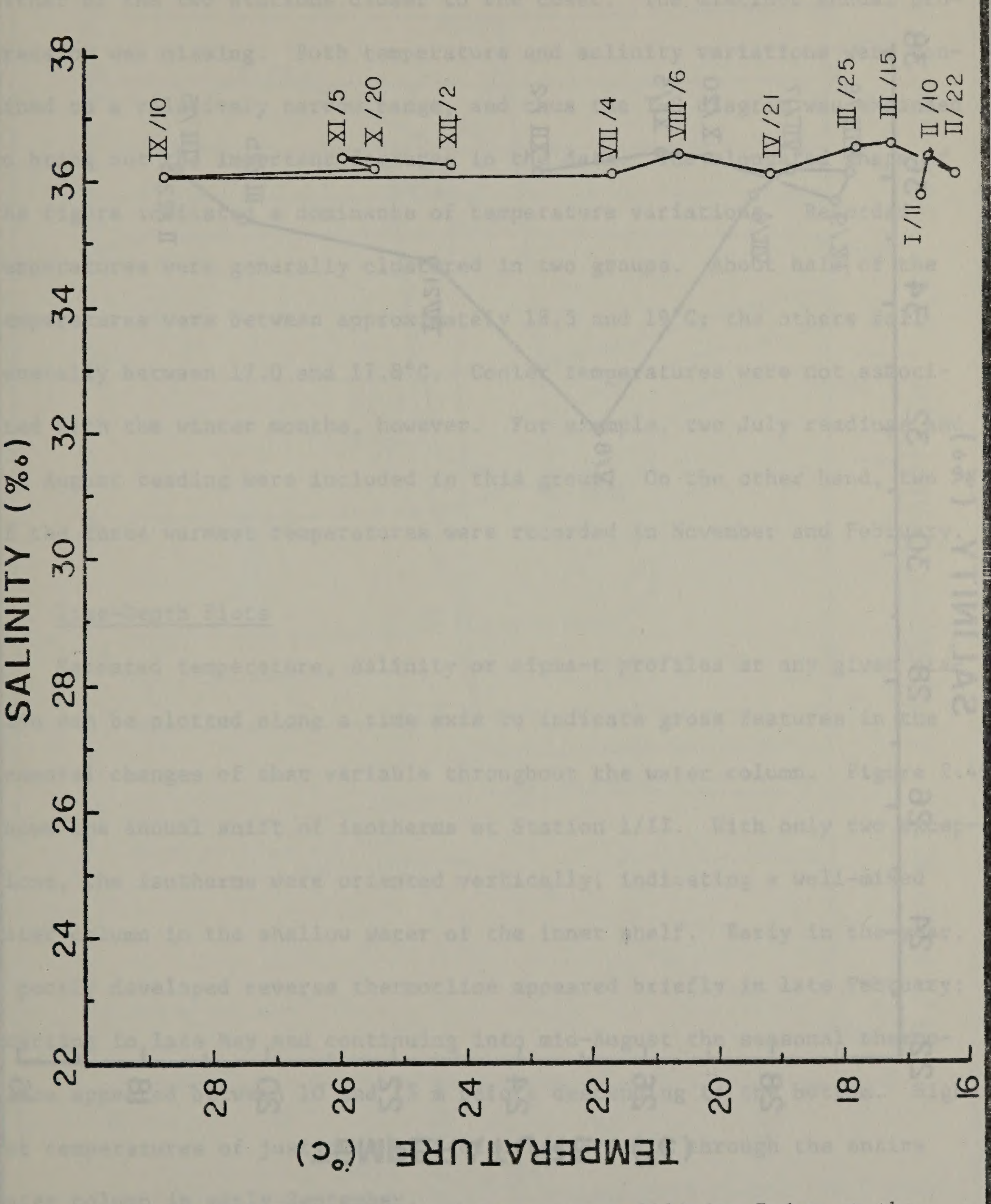

Figure 2.46 Annual Progression of Temperature-Salinity Pairs at the Bottom of Station 2/II.

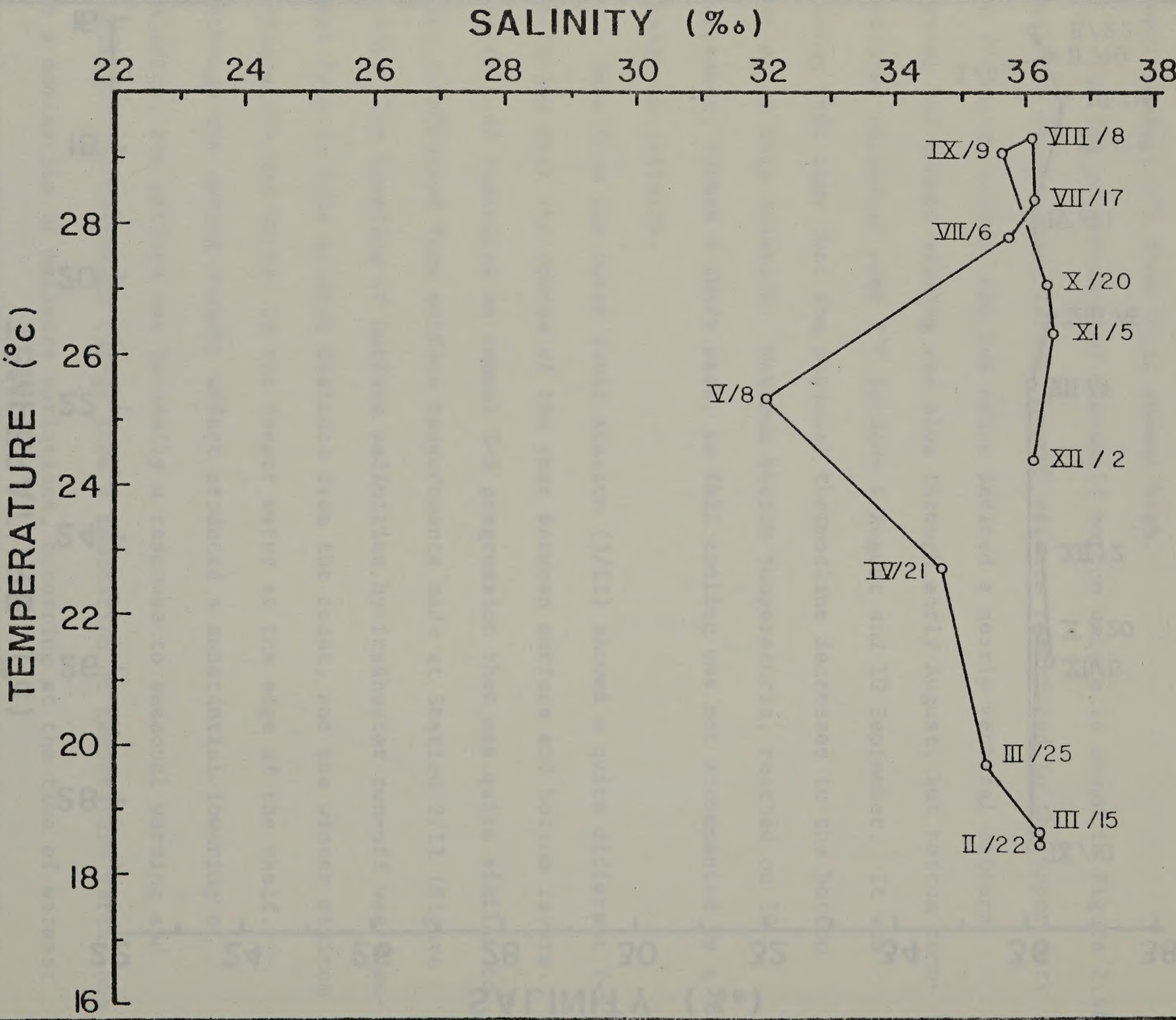

Figure 2.47 Annual Progression of Temperature-Salinity Pairs at the Surface at Station 3/II.

Bottom temperature and salinity data (Figure 2.48) presented a pattern that was significantly different from any of those representing conditions at the surface at Station 3/II, or at the surface or bottom at either of the two stations closer to the coast. The distinct annual progression was missing. Both temperature and salinity variations were confined to a relatively narrow range, and thus the T-S diagram was expanded to bring out the important features in the data. The elongated shape of the figure indicated a dominance of temperature variations. Recorded temperatures were generally clustered in two groups. About half of the temperatures were between approximately 18.5 and 19°C; the others fall generally between 17.0 and 17.8°C. Cooler temperatures were not associated with the winter months, however. For example, two July readings and one August reading were included in this group. On the other hand, two of the three warmest temperatures were recorded in November and February.

Time-Depth Plots

Repeated temperature, salinity or sigma-t profiles at any given station can be plotted along a time axis to indicate gross features in the temporal changes of that variable throughout the water column. Figure 2.49 shows the annual shift of isotherms at Station 1/II. With only two exceptions, the isotherms were oriented vertically, indicating a well-mixed water column in the shallow water of the inner shelf. Early in the year, a poorly developed reverse thermocline appeared briefly in late February; starting in late May and continuing into mid-August the seasonal thermocline appeared between 10 and 15 m before descending to the bottom. Highest temperatures of just over 29°C occurred briefly through the entire water column in early September.

Figure 2.50 shows the isohaline pattern constructed from salinity

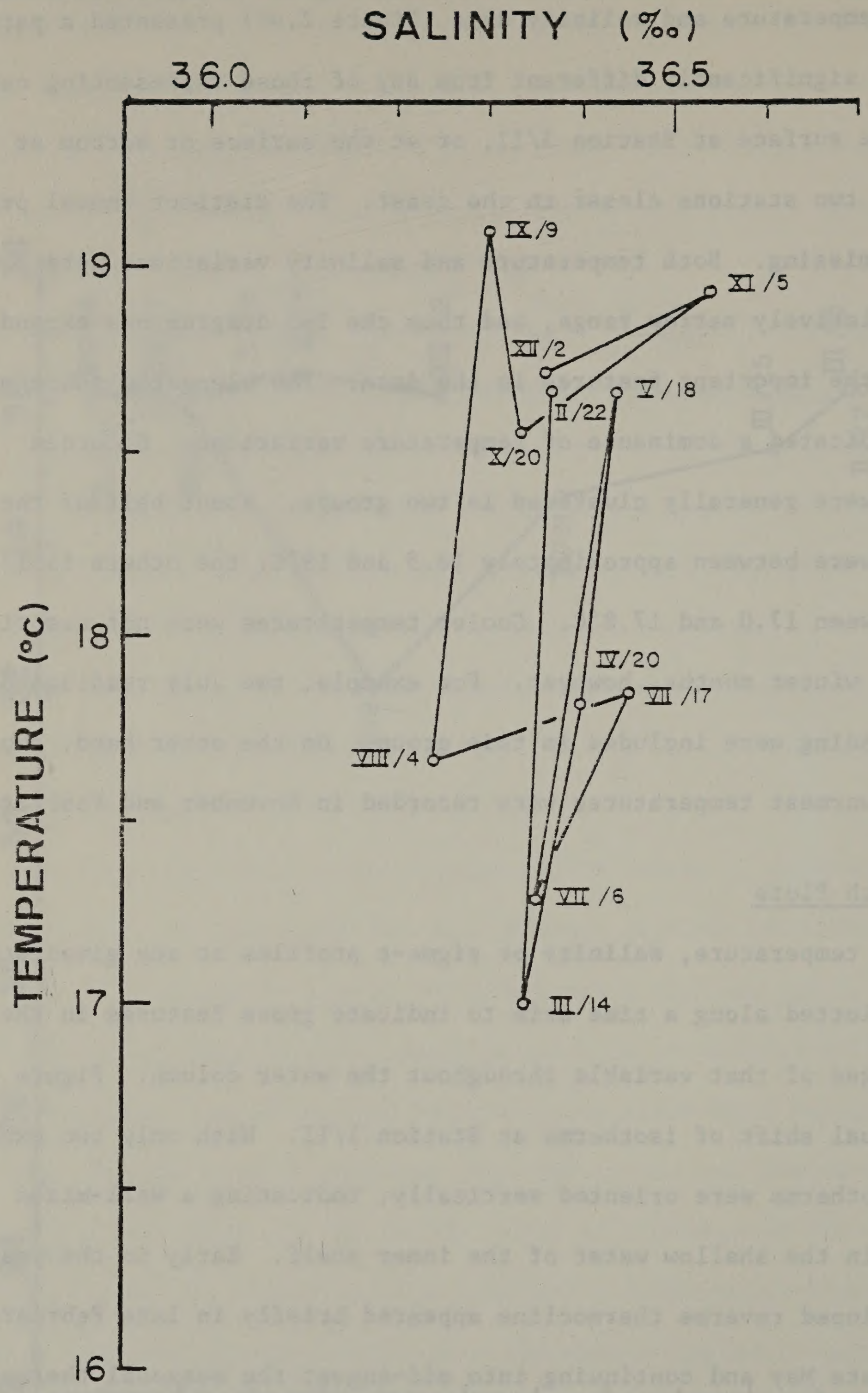

Figure 2.48 Annual Progression of Temperature-Salinity Pairs at the Bottom at Station 3/II.

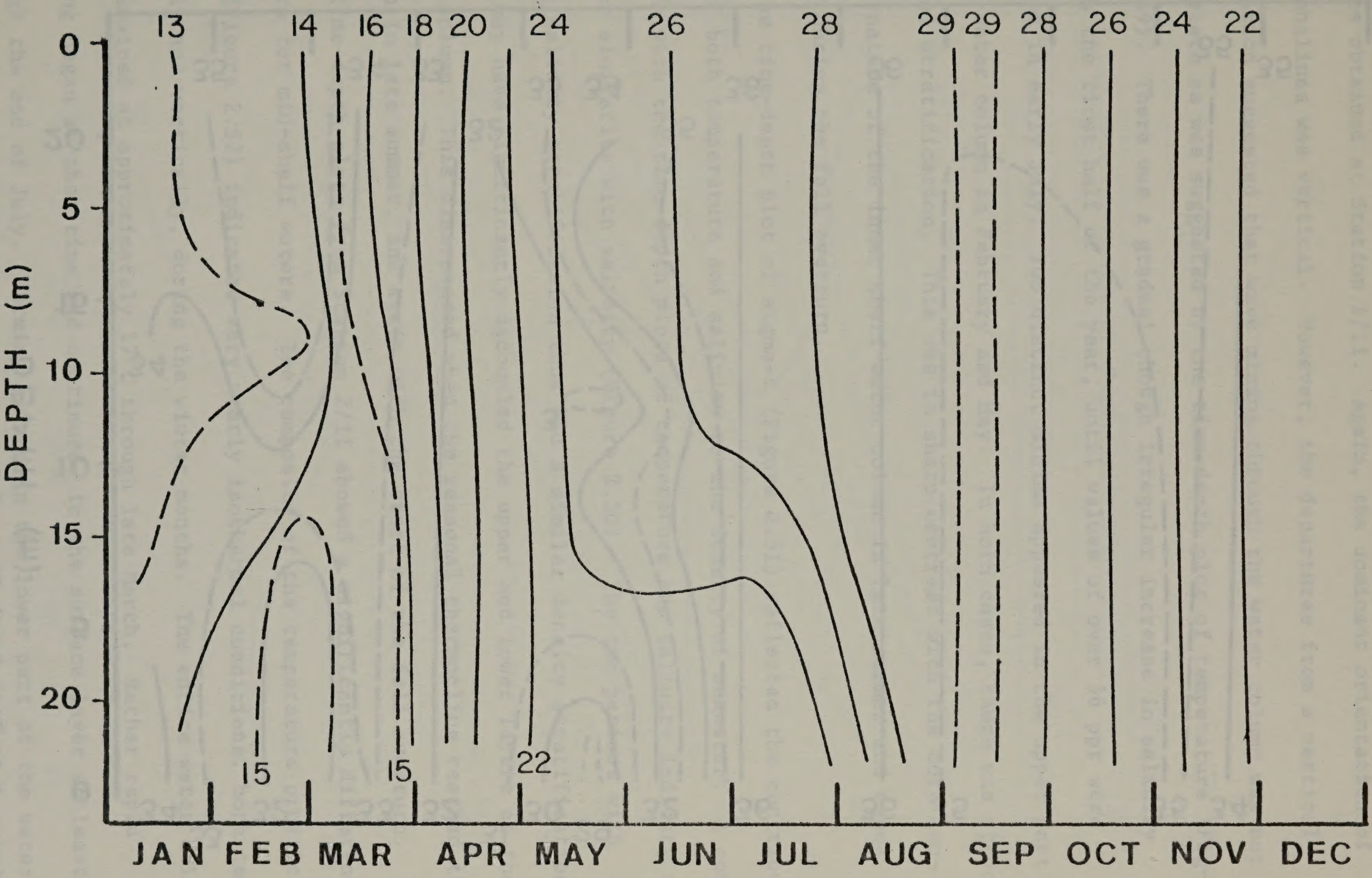

Figure 2.49 Time-Depth Plot of Temperature, in °C, at Station 1/II, 1977.

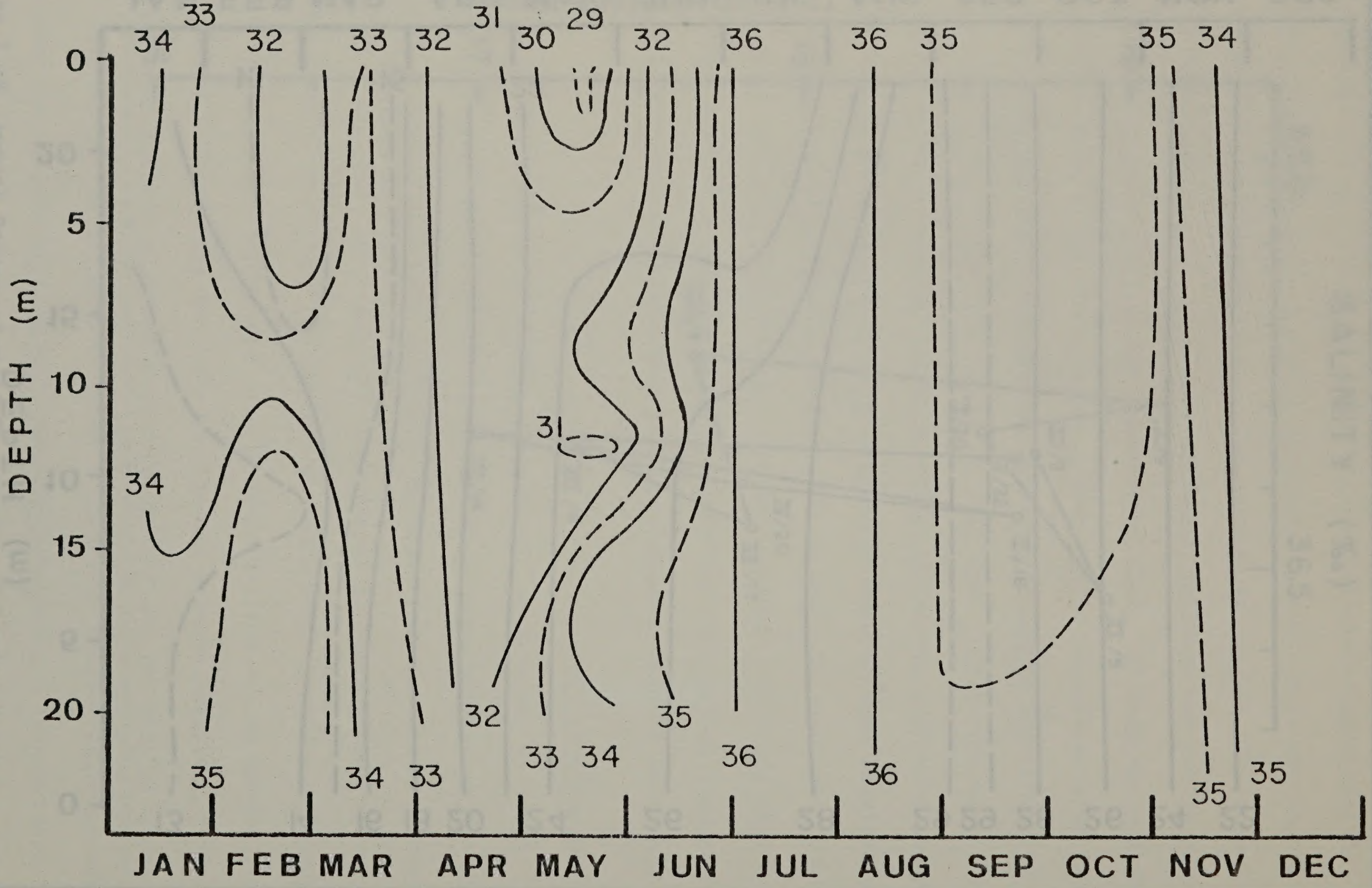

Figure 2.50 Time-Depth Plot of Salinity, in ppt, at Station 1/II, 1977.

profiles obtained at Station 1/II. Again, the dominant orientation of the isohalines was vertical. However, the departures from a vertical orientation suggested that wave mixing through the water column was not as complete as was suggested by the time-depth plot of temperature (Figure 2.49). There was a gradual though irregular increase in salinity through the first half of the year, until values of over 36 ppt were recorded in early July. Two distinct minima appeared in the upper part of the water column in February and May. In both cases, there was strong vertical stratification. This was in sharp contrast with the more isohaline nature of the inner shelf water column in late summer and especially during the fall overturn.

The time-depth plot of sigma-t (Figure 2.51) reflected the contribution of both temperature and salinity to the density of seawater. A comparison with the time-depth plots of temperature and salinity indicate a greater similarity with salinity (Figure 2.50). The two periods with strong salinity stratification thus had a similar density stratification which may have significantly decoupled the upper and lower layers of the water column. This disappeared when the seasonal thermocline reached the bottom in late summer, and again with the start of the fall overturn.

Time-depth plots from Station 2/II showed a significantly different pattern for mid-shelf waters. The composite of the temperature profile data (Figure 2.52) indicated very nearly isothermal conditions, both temporally and vertically, during the winter months. The entire water column remained at approximately 17°C through late March. Rather rapid warming began at that time and continued, in the surface layer at least, through the end of July. Warming slowed in the lower part of the water column almost immediately, causing a distinct slope in the isotherms through mid-September. Highest mid-shelf temperatures of just over 29°C,

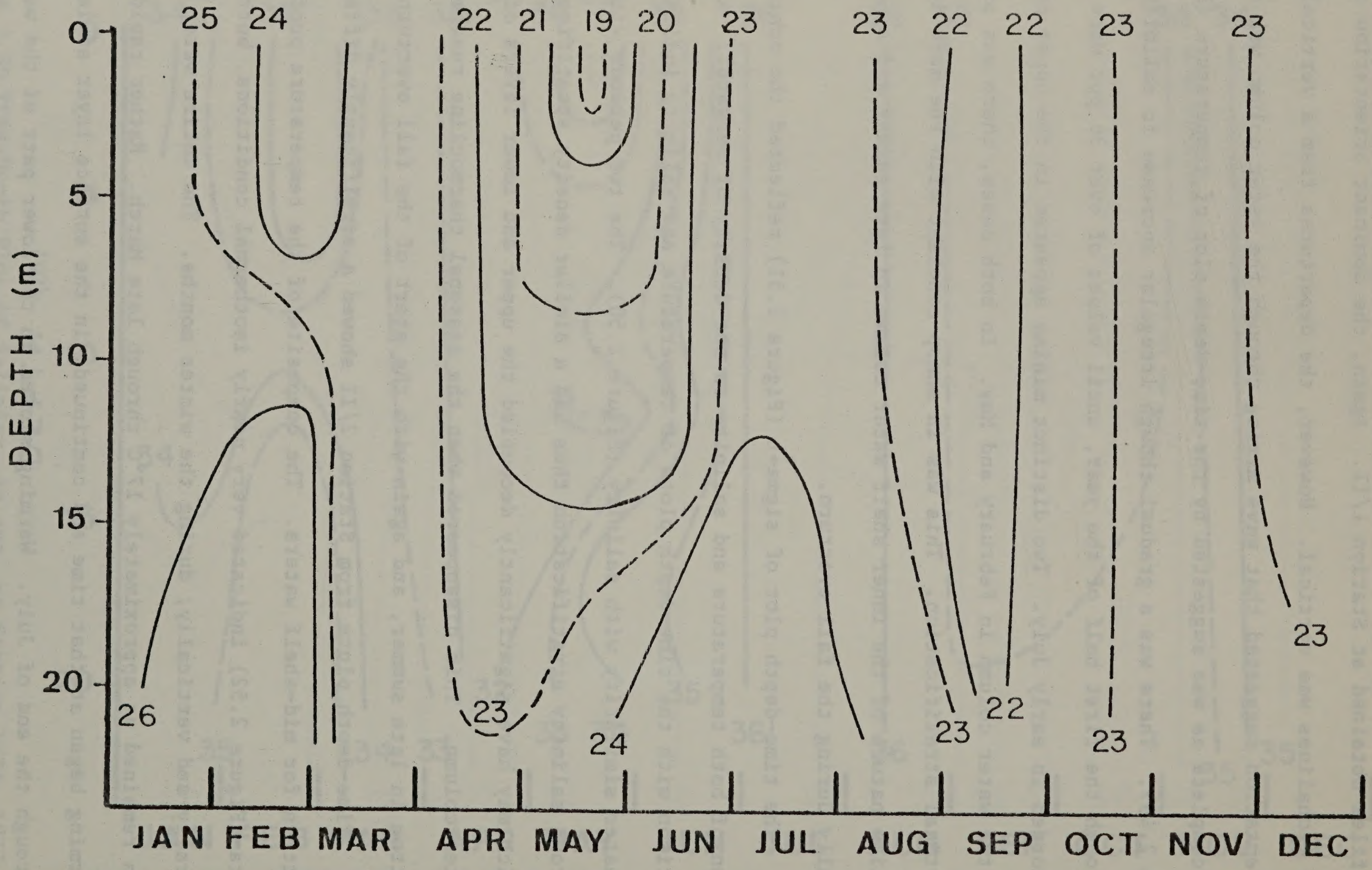

Figure 2.51 Time-Depth Plot of Sigma-t at Station 1/II, 1977.

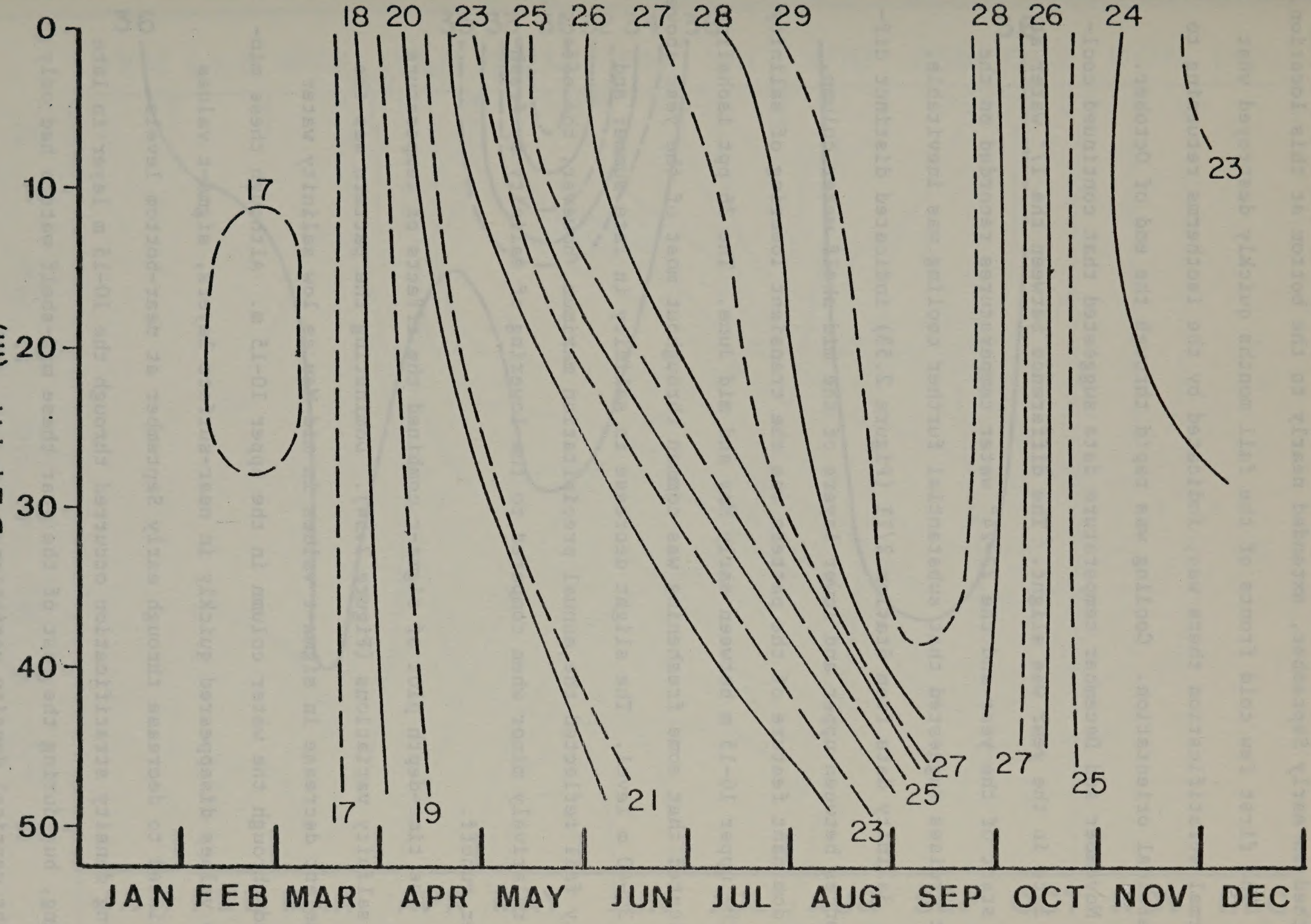

Figure 2.52 Time-Depth Plot of Temperature, in °C, at Station 2/II, 1977.

reached in early September, extended nearly to the bottom at this location.

The first few cold fronts of the fall months quickly destroyed what thermal stratification there was, indicated by the isotherms returning to a vertical orientation. Cooling was rapid through the end of October. The November and December temperature data suggested that continued cooling late in the year was slight. The difference between the 17° water at the start of the year and the 23-24° water temperatures recorded on the last cruises suggested that substantial further cooling was inevitable.

Salinity data from Station 2/II (Figure 2.53) indicated distinct differences between upper and lower layers of the mid-shelf water column. The dominant feature of the pattern was the transient lowering of salinity in the upper 10-15 m between early May and mid June. The 36 ppt isohaline indicated that some freshening was common throughout most of the year above the 30-40 m level. The slight decrease in salinity in late summer and early fall reflected the annual precipitation maximum, however, the effect was relatively minor when compared to the lowering of salinity by freshwater runoff.

The time-depth plot of sigma-t combined the effects of temperature and salinity variations (Figure 2.54). Dominating the pattern was the transient decrease in sigma-t values in mid-May as low salinity water moved through the water column in the upper 10-15 m. Although these minimum values disappeared quickly in near-surface layers, sigma-t values continued to decrease through early September at near-bottom levels. Strong density stratification occurred through the 10-15 m layer in late spring, but during the rest of the year these mid-shelf waters had only slight vertical density variations.

Figure 2.55 shows the time-depth plot of water column temperatures from Station 3/II. Following a nearly three-month period of near homo-

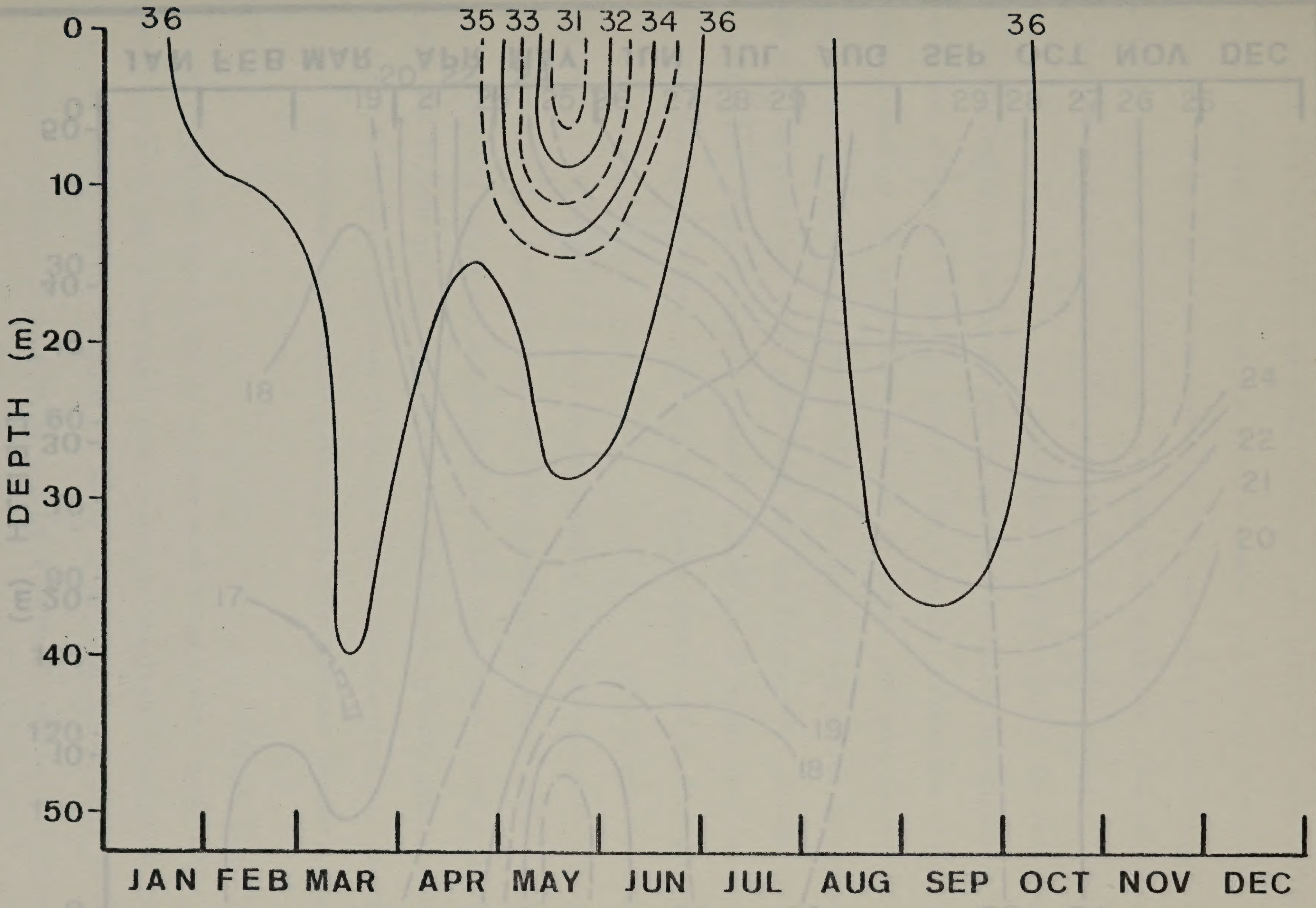

Figure 2.53 Time-Depth Plot of Salinity, in ppt, at Station 2/II, 1977.

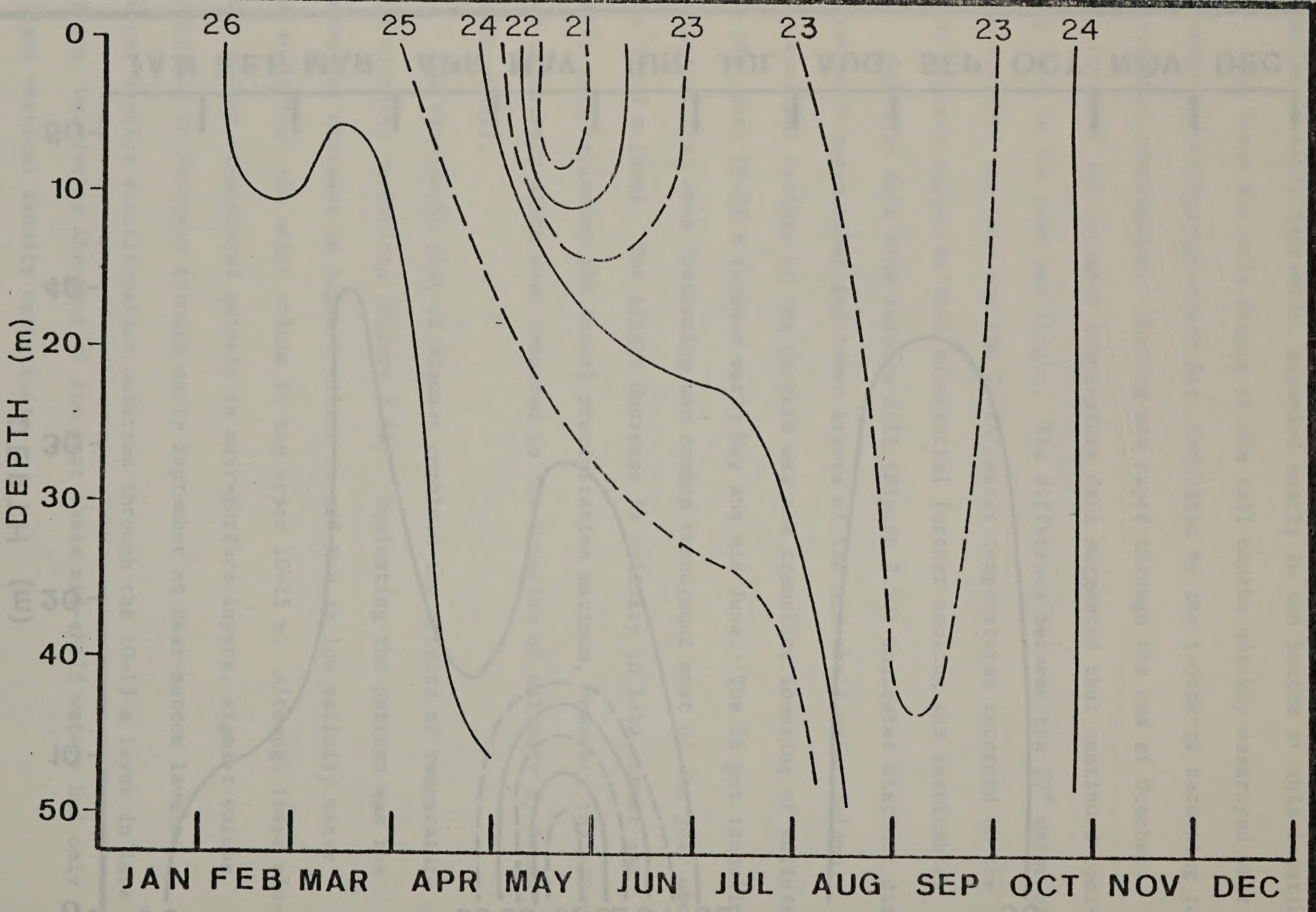

Figure 2.54 Time-Depth Plot of Sigma-t at Station 2/II, 1977.

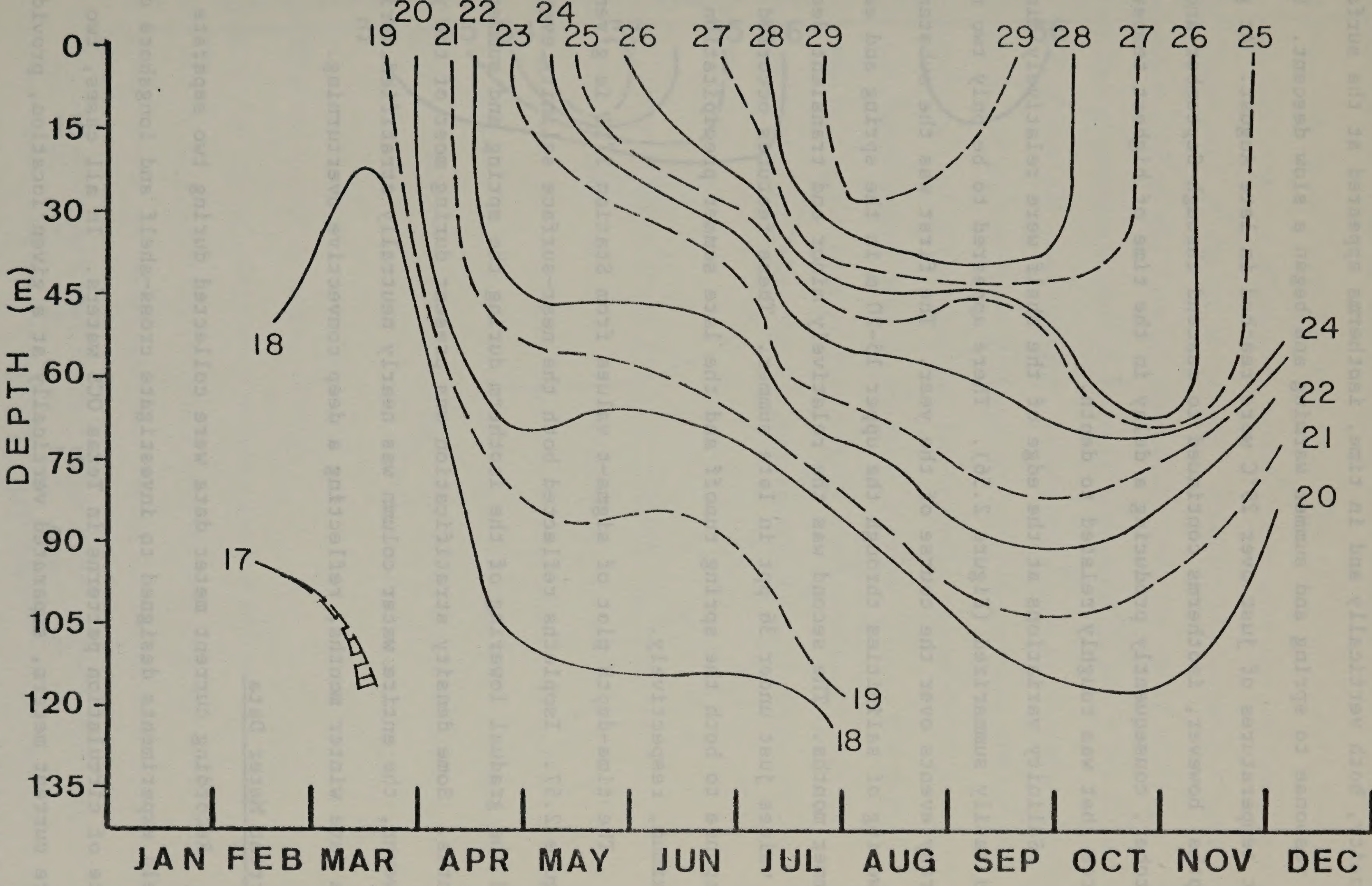

Figure 2.55 Time-Depth Plot of Temperatures, in °C, at Station 3/II, 1977.

geneity, both vertically and in time, isotherms appeared at the surface in response to spring and summer warming and began a slow descent. Highest temperatures of just over 29°C were reached in late August. At greater depths, however, isotherms continued to descend through September and into October, consequently producing a delay in the time of highest temperatures that was roughly related to depth.

Salinity variations at the edge of the shelf were relatively minor and easily summarized (Figure 2.56). There appeared to be only two noteworthy events over the course of the year. The first was the substantial lowering of salinities through the upper 15-20 m in the spring and early summer months. The second was the relatively minor and transient decrease to values just under 36 ppt in late summer. These features occurred in response to both the spring runoff and the late summer precipitation maximum, respectively.

The time-depth plot of sigma-t values from Station 3/II is given in Figure 2.57. Isopleths reflected both the near-surface salinity events and the gradual lowering of the isotherm during the spring and summer months. Some density stratification was present during most of the year, however, the entire water column was nearly neutrally stratified during the late winter months, reflecting a deep convective overturning.

Current Meter Data

Recording current meter data were collected during two separate field experiments designed to investigate cross-shelf and longshore coherence of circulation patterns in Texas OCS waters. In all cases, two or more current meters, separated vertically at a given location, provided additional information on vertical coherence in shelf motion.

Continental shelf circulation is characteristically complex. The

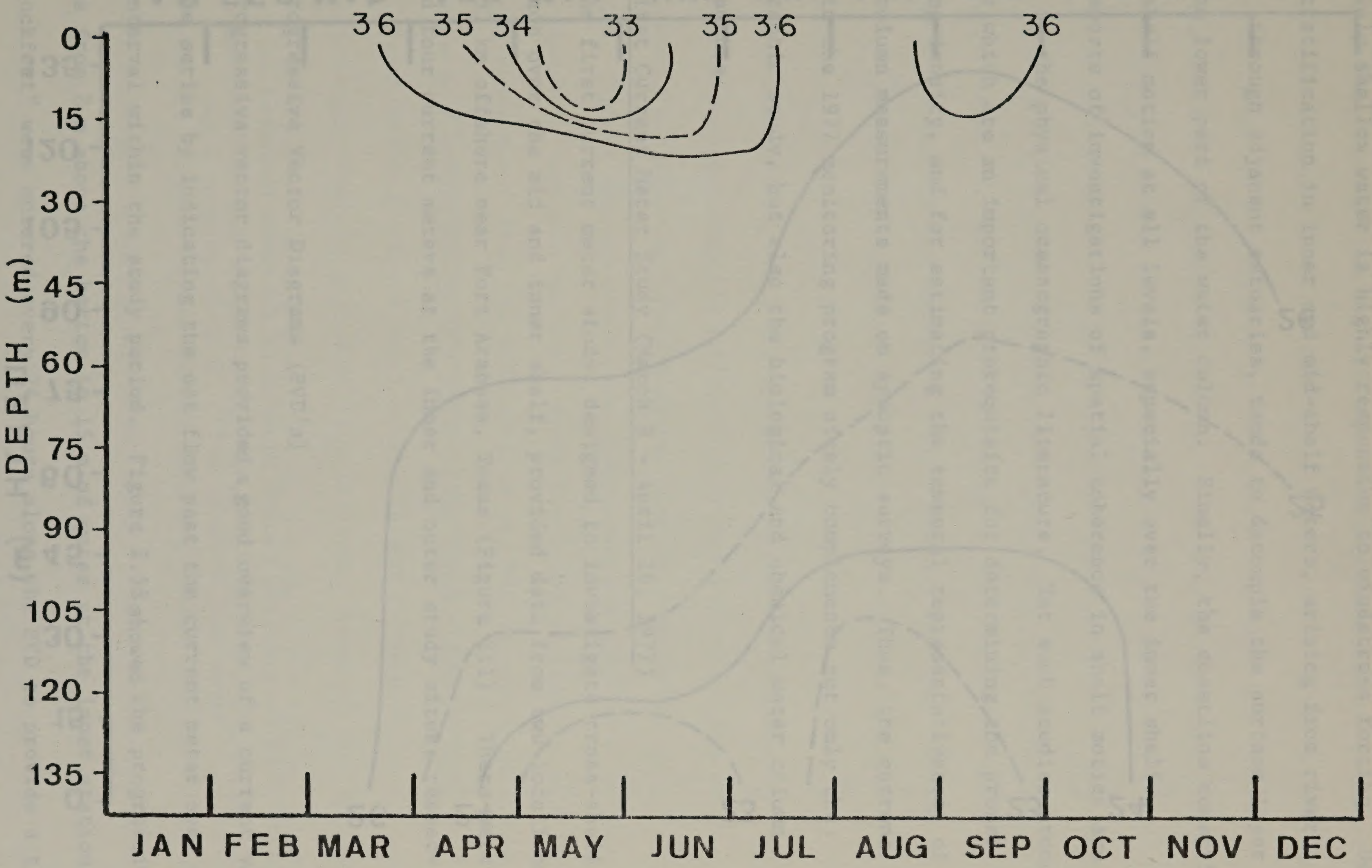

Figure 2.56 Time-Depth Plot of Salinity, in ppt, at Station 3/II, 1977.

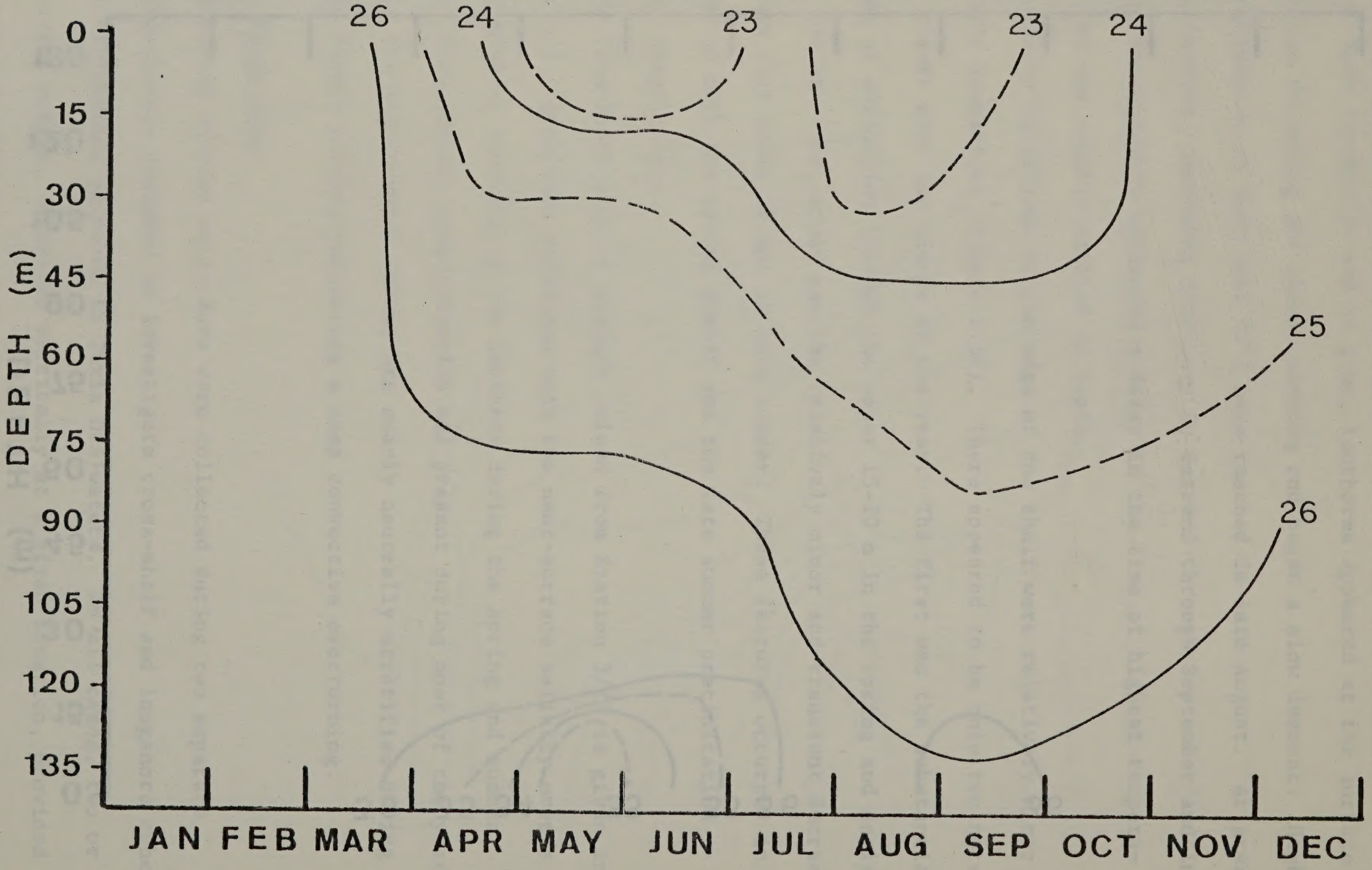

Figure 2.57 Time-Depth Plot of Sigma-t at Station 3/II, 1977.

relatively shallow water is highly responsive to windstress forcing. Density stratification in inner and mid-shelf waters, arising from river runoff through adjacent estuaries, tends to decouple the surface layer from the lower part of the water column. Finally, the coastline constricts cross-shelf motion at all levels, especially over the inner shelf.

Reports of investigations of spatial coherence in shelf motion are sparse in the physical oceanographic literature. Yet such studies produce results which are an important prerequisite for determining the proper sampling density, and for estimating the temporal representativeness of water column measurements made on synoptic surveys. Thus, the current study in the 1977 monitoring program nicely complements not only the hydrographic study, but also the biological and chemical water column measurements.

First Current Meter Study (March 9 - April 26, 1977)

The first current meter study, designed to investigate cross-shelf coherence over the mid and inner shelf, provided data from two locations, 6 and 33 km offshore near Port Aransas, Texas (Figure 2.1). There were two and four current meters at the inner and outer study sites, respectively.

Progressive Vector Diagrams (PVD's)

Progressive vector diagrams provided a good overview of a current vector time series by indicating the net flow past the current meter over any time interval within the study period. Figure 2.58 showed the progressive vectors from 2 m above the bottom in 15 m of water at the inner station. The "duckfeet" were entered every 24 hours along the PVD to provide a time reference. The sampling site was approximately 6 km offshore along the shoreward extension of Transect II. The PVD indicates there was a some-

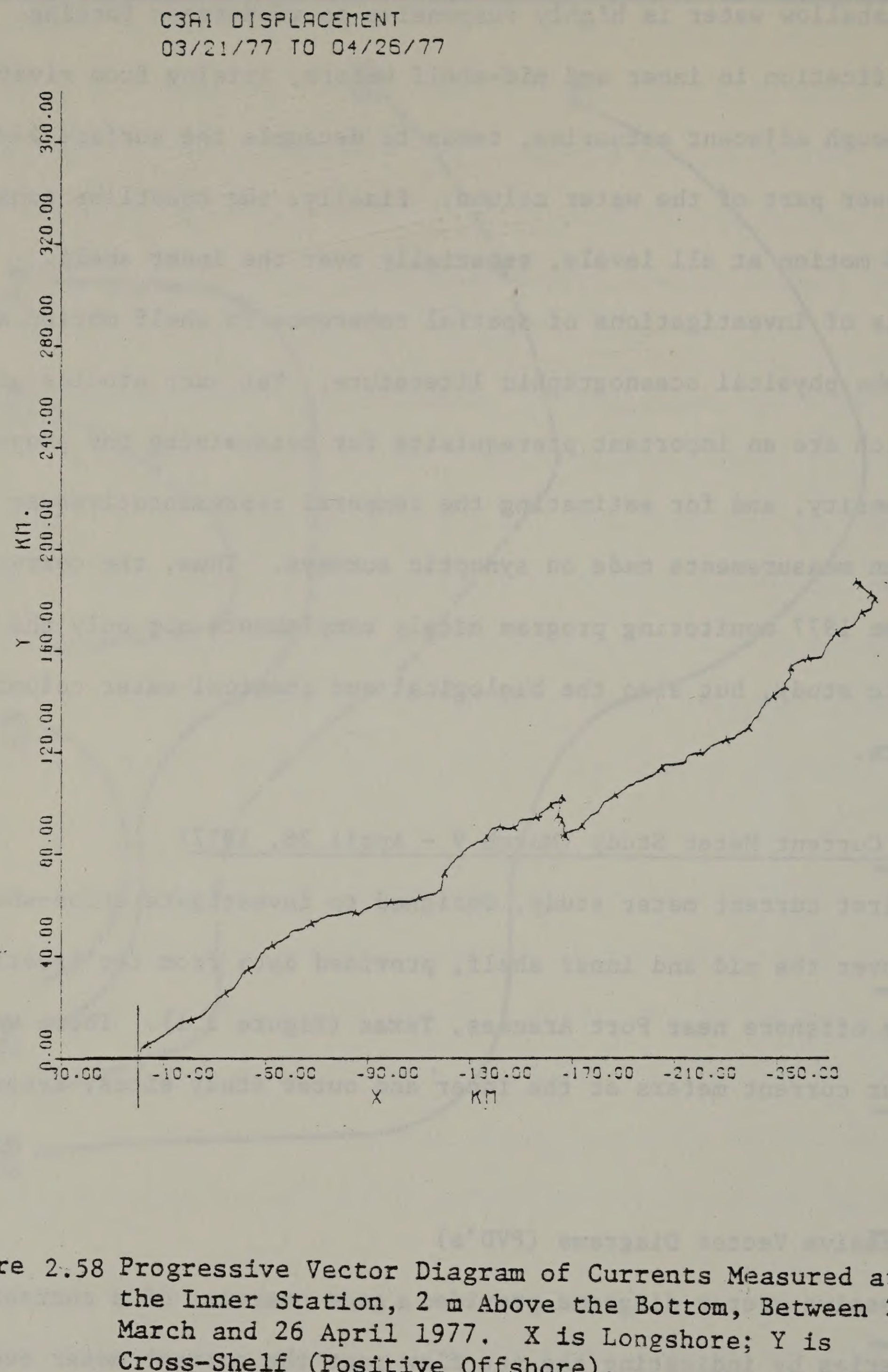

Figure 2.58 Progressive Vector Diagram of Currents Measured at the Inner Station, 2 m Above the Bottom, Between 21 March and 26 April 1977. X is Longshore; Y is Cross-Shelf (Positive Offshore).

what meandering near-bottom current with a distinct offshore component. Over the approximately 36-day study period, 281.2 km of water moved past the current meter in a longshore (x-component) direction and 190 km in the cross-shelf (y-component) direction. From this a resultant current vector having a magnitude of 10.8 cm/sec and a direction of 179°, or 34° to the left (offshore) of the local coastline was calculated. The pattern was characterized by a more or less steady flow along a southerly heading, with slight meanders, and one interval of about four days when the flow was halted and the current assumed a nearly onshore heading. At the very end of the record, the longshore component of the current reversed for the first and only time.

At the upper level, 10 m off the bottom, currents recorded at the inner station showed distinctly different characteristics (Figure 2.59). The PVD showed a current pattern which was tightly constrained by the local coastline. Midway through the record, there was a significant reversal in the nearshore current, but the current continued to closely parallel the local coastline. From the endpoints of the PVD, a resultant current velocity with a magnitude of 13.8 cm/sec, and a direction of 214°, of 1° to the right (onshore) of the local coastline was calculated.

During approximately this same time interval, currents were monitored at the outer station. At the lowest level, 5 m above the bottom, the PVD showed a pattern that was clearly constrained by the local coastline and/or bottom contours (Figure 2.60). The pattern was one of longshore flow interrupted at three points during the 48-day study. The first two periods involved a reversal in longshore motion, while the third indicated a change to predominantly onshore flow. It was of interest to note that this occurred at the same time the flow was largely in an offshore direction at the lower level at the inner station. The bathymetric constraint on the near-

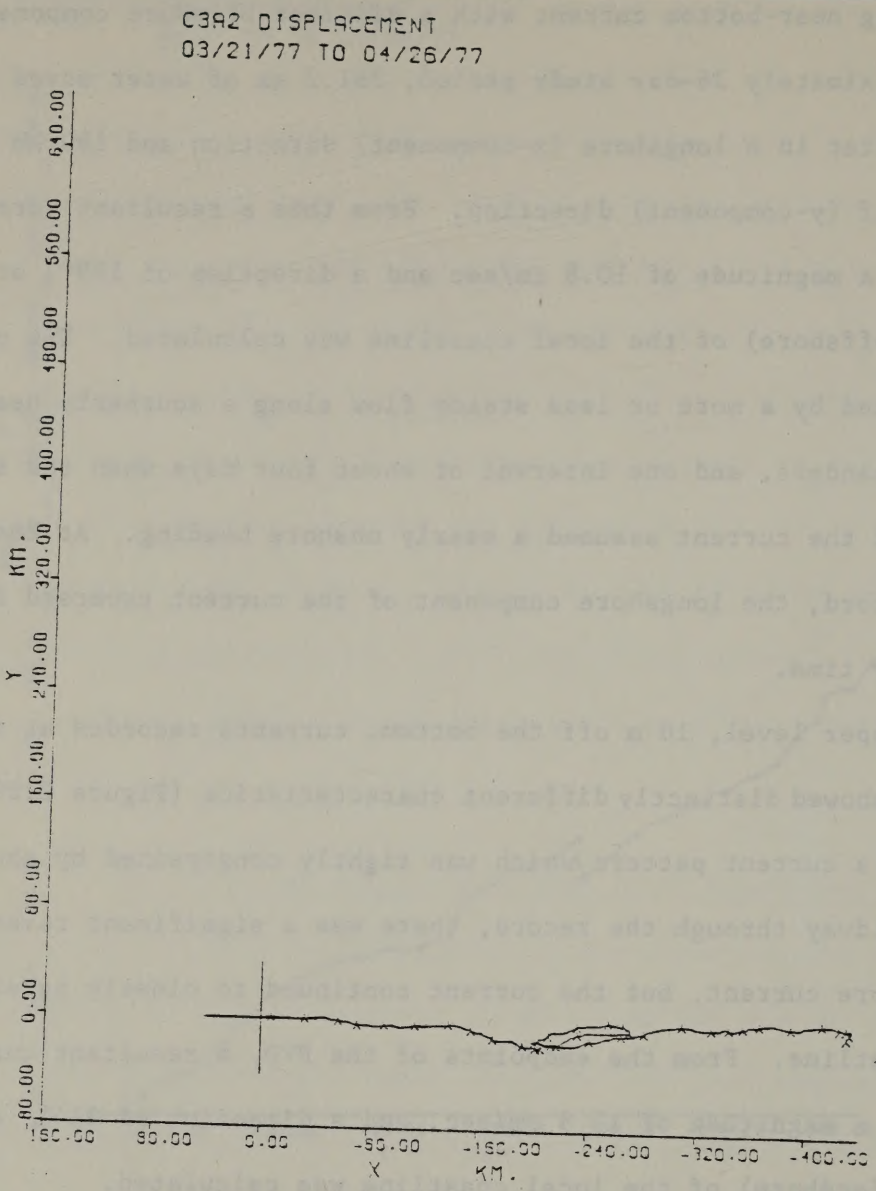

Figure 2.59 Progressive Vector Diagram of Currents Measured at the Inner Station, 10 m Above the Bottom, Between 21 March and 26 April 1977. X is Longshore; Y is Cross-Shelf (Positive Offshore).

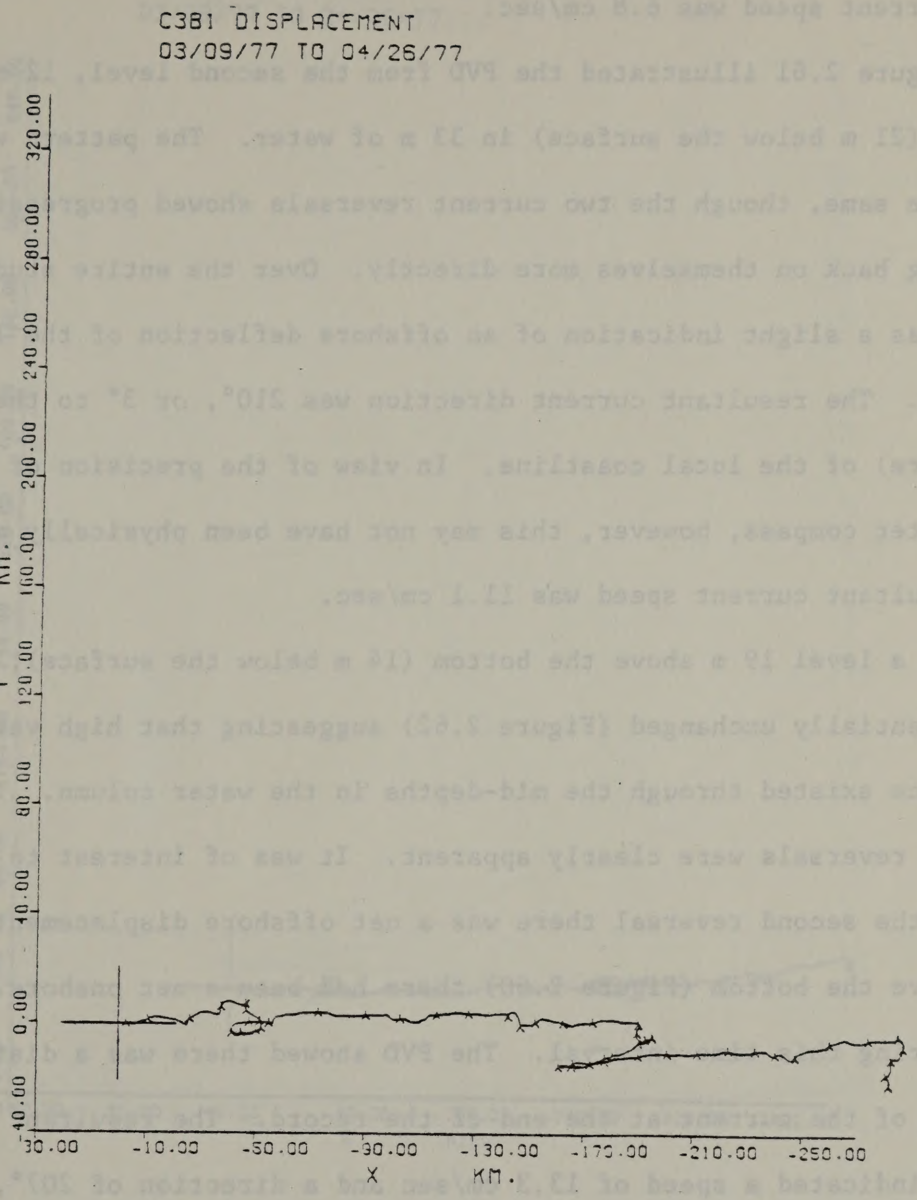

Figure 2.60 Progressive Vector Diagram of Currents Measured at the Outer Station, 5 m Above the Bottom, Between 9 March and 26 April 1977. X is Longshore; Y is Cross-Shelf (Positive Offshore).

shore flow was clearly demonstrated by the resultant current direction of 217°, or just 4° to the right (onshore) of the local coastline. The resultant current speed was 6.8 cm/sec.

Figure 2.61 illustrated the PVD from the second level, 12 m above the bottom (21 m below the surface) in 33 m of water. The pattern was basically the same, though the two current reversals showed progressive vectors doubling back on themselves more directly. Over the entire study period, there was a slight indication of an offshore deflection of the longshore current. The resultant current direction was 210°, or 3° to the left (offshore) of the local coastline. In view of the precision of the current meter compass, however, this may not have been physically meaningful. The resultant current speed was 11.1 cm/sec.

At a level 19 m above the bottom (14 m below the surface), the PVD was essentially unchanged (Figure 2.62) suggesting that high vertical coherence existed through the mid-depths in the water column. The two current reversals were clearly apparent. It was of interest to note that during the second reversal there was a net offshore displacement, while 5 m above the bottom (Figure 2.60) there had been a net onshore displacement during this time interval. The PVD showed there was a distinct slowing of the current at the end of the record. The resultant current vector indicated a speed of 13.3 cm/sec and a direction of 207°, or 6° to the left (offshore) of the local coastline.

A distinctly different pattern was indicated for the uppermost current meter time series, 26 m above the bottom and 7 m below the surface (Figure 2.63). The two periods during which the current reversed lower in the water column were indicated at this level by a slowing of a predominantly longshore current with a distinctly offshore component. Instead

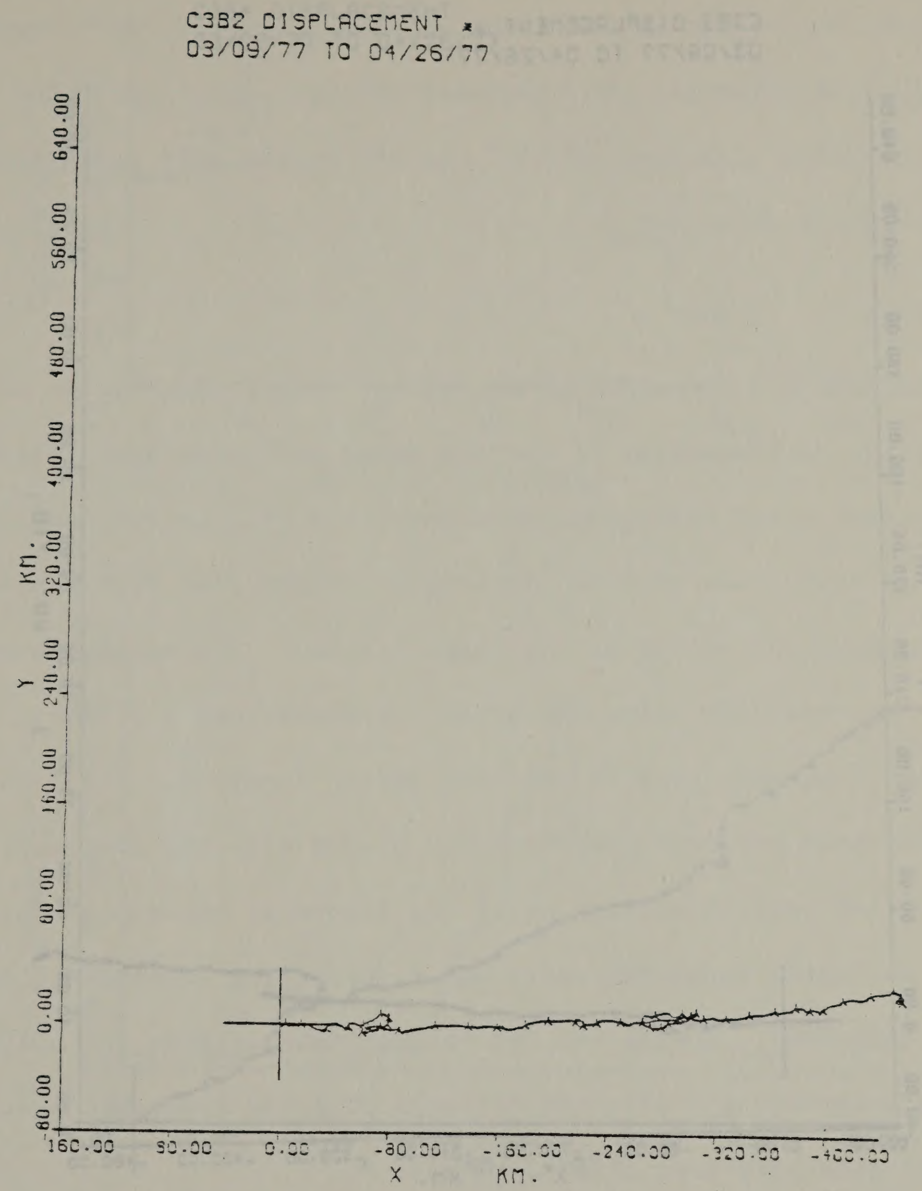

Figure 2.61 Progressive Vector Diagram of Currents Measured at the Outer Station, 12 m Above the Bottom, Between 9 March and 26 April 1977. X is Longshore; Y is Cross-shelf (Positive Offshore).

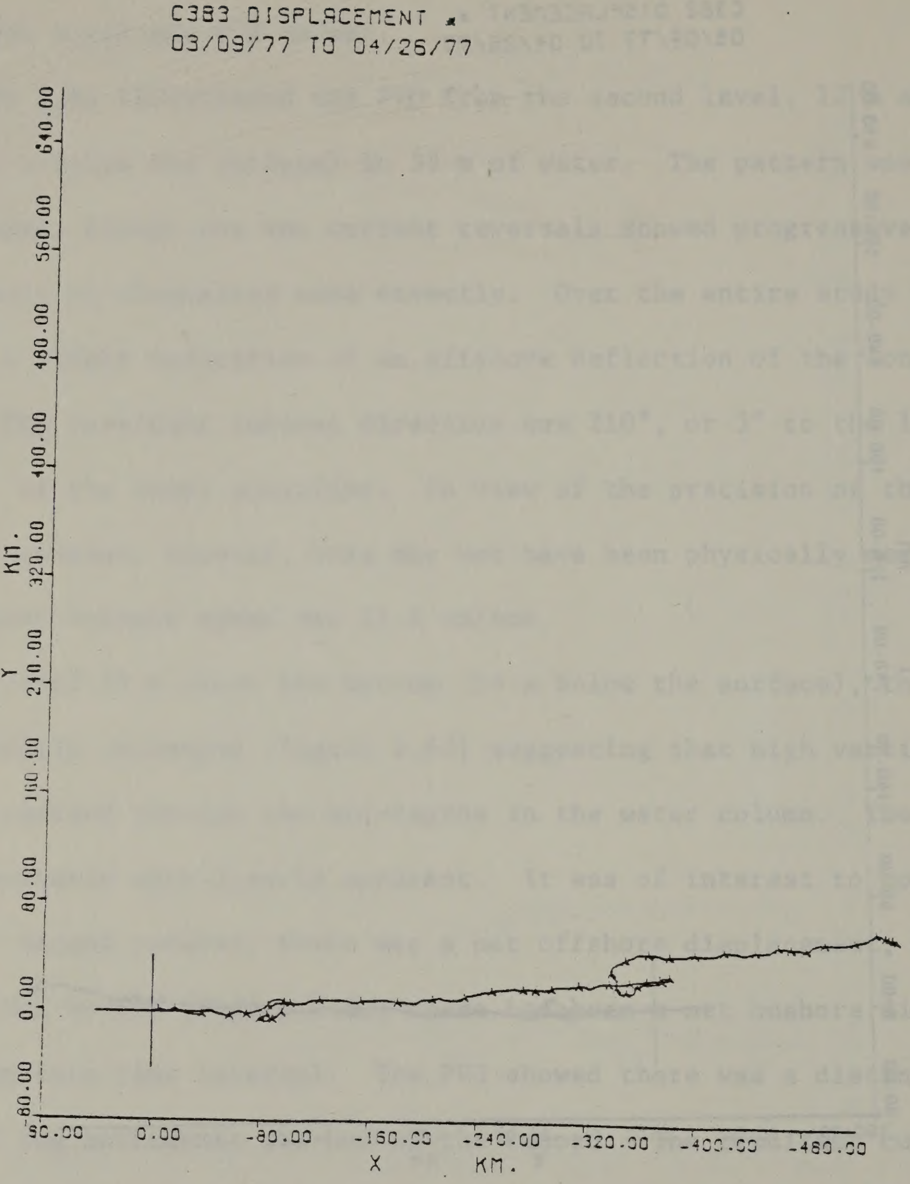

Figure 2.62 Progressive Vector Diagram of Currents Measured at the Outer Station, 19 m Above the Bottom, Between 9 March and 26 April 1977. X is Longshore; Y is Cross-shelf (Positive Offshore).

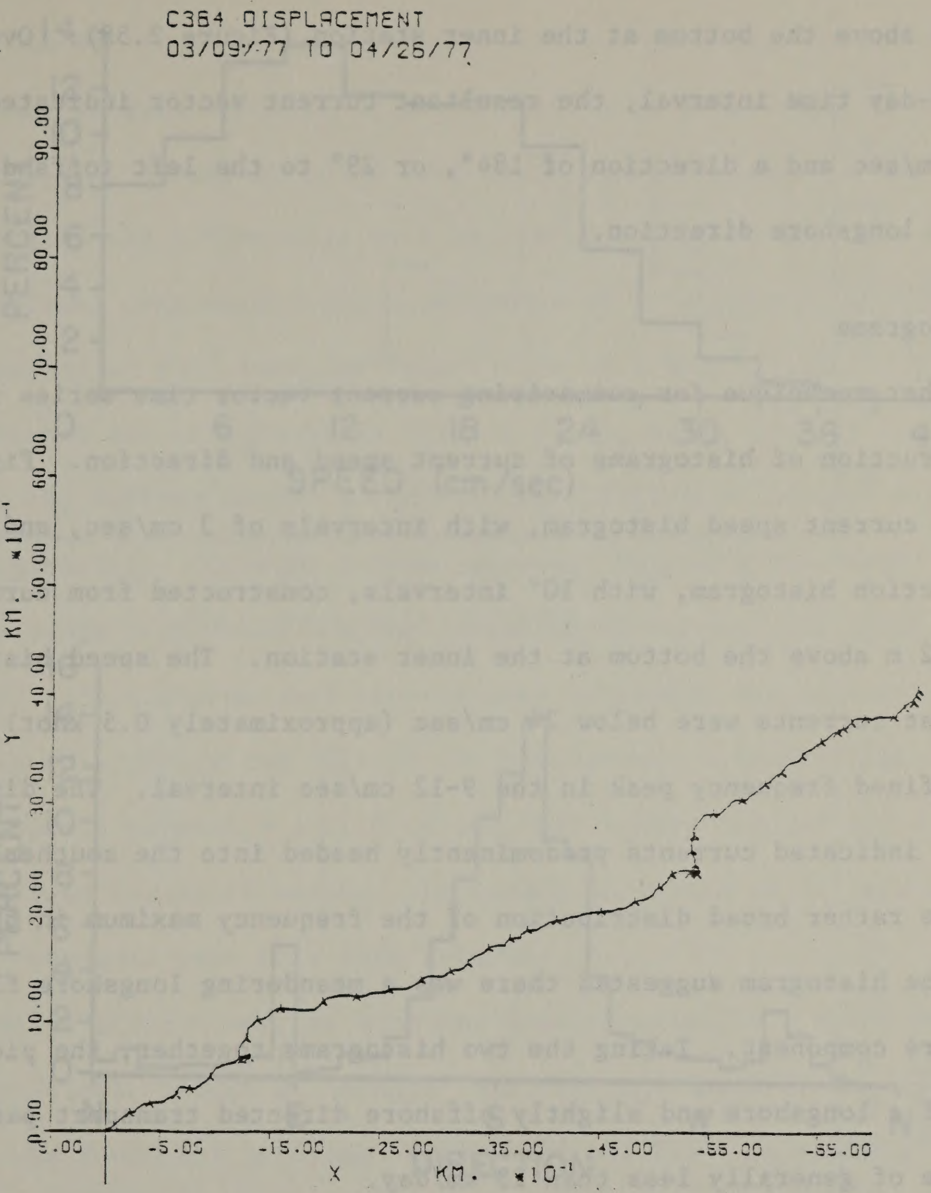

Figure 2.63 Progressive Vector Diagram of Currents Measured at the Outer Station, 26 m Above the Bottom, Between 9 March and 26 April 1977. X is Longshore; Y is Cross-Shelf (Positive Offshore).

of a current direction constrained by the coastline, the PVD showed that a current pattern with a strong offshore component existed much like that found 2 m above the bottom at the inner station (Figure 2.58). Over the entire 48-day time interval, the resultant current vector indicated a speed of 20.5 cm/sec and a direction of 184°, or 29° to the left (offshore) of the local longshore direction.

Histograms

Another technique for summarizing current vector time series involved the construction of histograms of current speed and direction. Figure 2.64 shows the current speed histogram, with intervals of 3 cm/sec, and the current direction histogram, with 10° intervals, constructed from currents measured 2 m above the bottom at the inner station. The speed histogram showed most currents were below 24 cm/sec (approximately 0.5 knot), with a poorly defined frequency peak in the 9-12 cm/sec interval. The direction histogram indicated currents predominantly headed into the southerly quadrant. The rather broad distribution of the frequency maximum in this portion of the histogram suggested there was a meandering longshore flow with an offshore component. Taking the two histograms together, the picture was one of a longshore and slightly offshore directed transport past the study site of generally less than 23 km/day.

At the upper level at the inner station (Figure 2.65) significantly stronger currents were recorded, and the flow more closely paralleled the coastline. The speed histogram had a twin peak within the range of 12-21 cm/sec, and frequencies decreased only slowly with increasing speed. Most observations were less than 47 cm/sec, indicating daily transports of 40 km or less, characteristic of this time interval. The direction histogram showed a concentration of current headings between 190 and 250°.

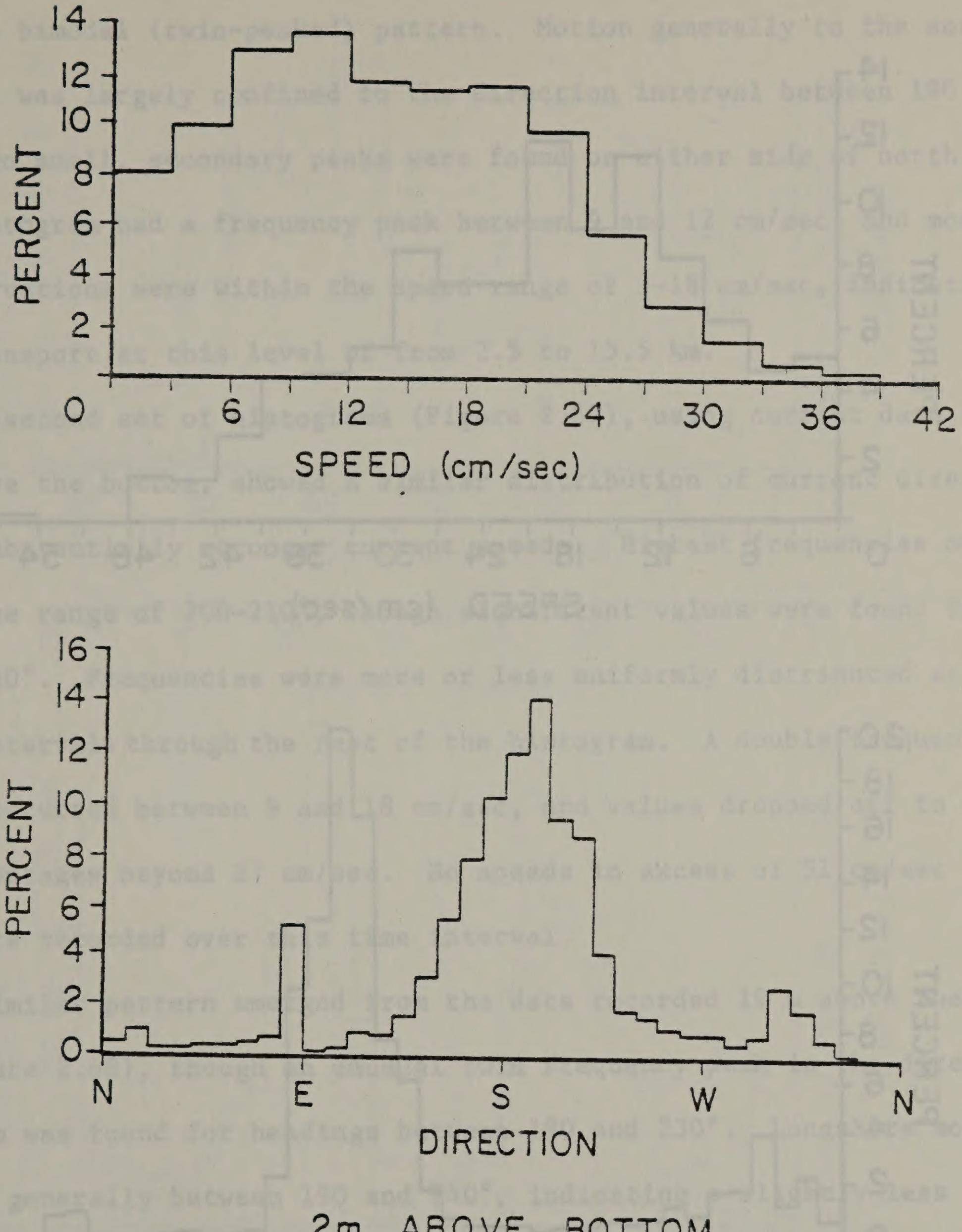

Figure 2.64 Histograms of Current Speed (3 cm/sec intervals) and Current Direction (10° intervals), 2 m Above Bottom at Inner Station, 21 March through 26 April 1977.

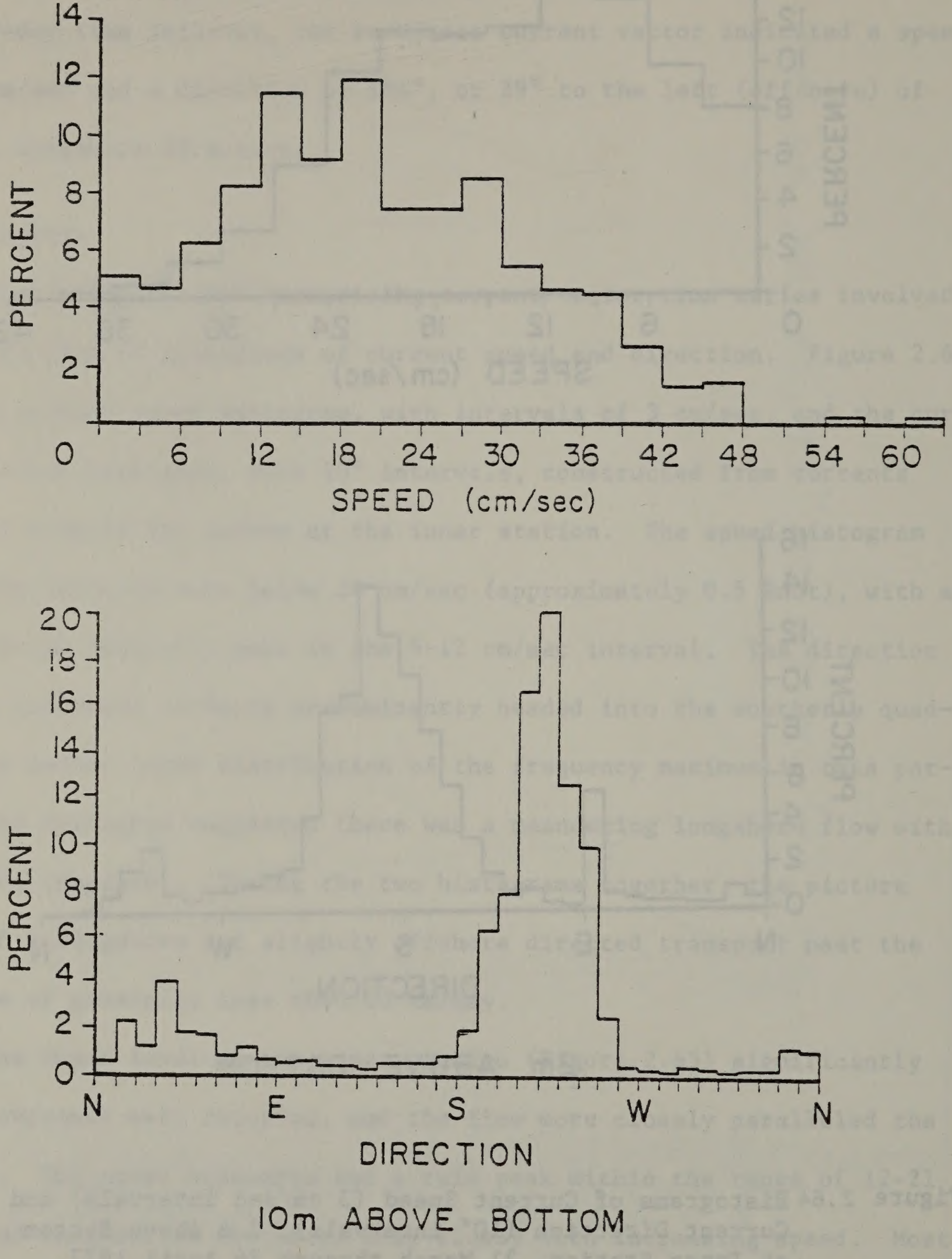

Figure 2.65 Histograms of Current Speed (3 cm/sec intervals) and Current Direction (10° intervals), 10 m Above Bottom, at Inner Station, 21 March through 26 April 1977.

At the lowest level at the outer station (Figure 2.66), current speeds were significantly slower, and the direction histogram indicated more of a bimodal (twin-peaked) pattern. Motion generally to the south-southwest was largely confined to the direction interval between 190 and 240°. Two small, secondary peaks were found on either side of north. The speed histogram had a frequency peak between 9 and 12 cm/sec, and most of the observations were within the speed range of 3-18 cm/sec, indicating a daily transport at this level of from 2.5 to 15.5 km.

The second set of histograms (Figure 2.67), using current data from 12 m above the bottom, showed a similar distribution of current directions, but at substantially stronger current speeds. Highest frequencies occurred within the range of 200-210°, though significant values were found from 190 to 260°. Frequencies were more or less uniformly distributed at about 1%/10° intervals through the rest of the histogram. A double frequency maximum occurred between 9 and 18 cm/sec, and values dropped off to quite low percentages beyond 27 cm/sec. No speeds in excess of 51 cm/sec (one knot) were recorded over this time interval.

A similar pattern emerged from the data recorded 19 m above the bottom (Figure 2.68), though an unusual twin frequency peak in the direction histogram was found for headings between 190 and 230°. Longshore motion occurred generally between 190 and 240°, indicating a slightly less meandering current at this level in the water column. The speed histogram showed a peak within the range of 12-15 cm/sec, and a shift to somewhat higher values. No current speeds greater than 48 cm/sec were observed.

The observed histogram from the upper most level, 26 m above the bottom and nominally 6 m below the surface, was distinctly shifted counterclockwise into a more southerly direction (Figure 2.69). Highest frequencies occurred generally between 160 and 230°. Frequencies were small else-

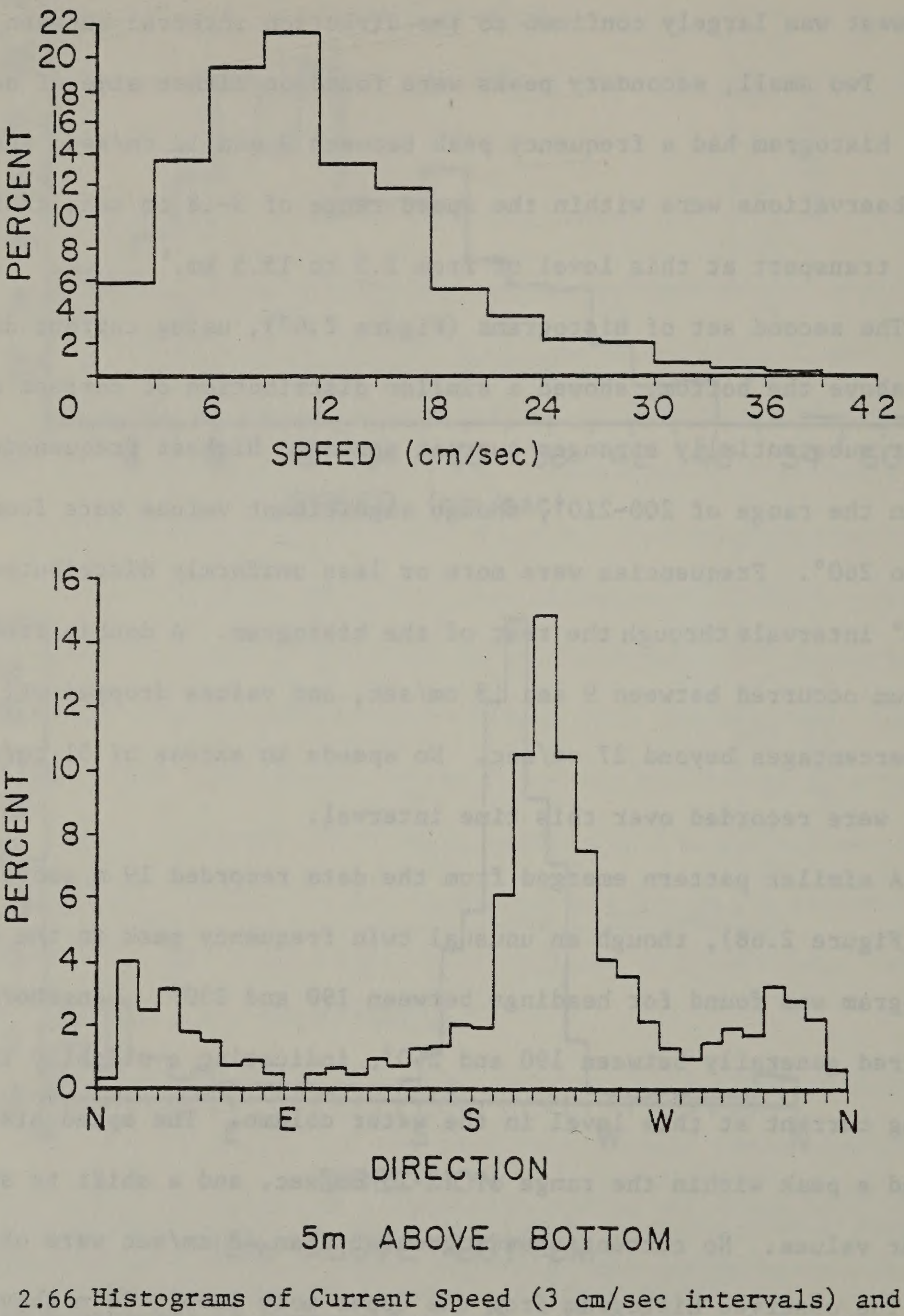

Figure 2.66 Histograms of Current Speed (3 cm/sec intervals) and Current Direction (10° intervals) 5 m Above Bottom, at Outer Station, 9 March through 26 April 1977.

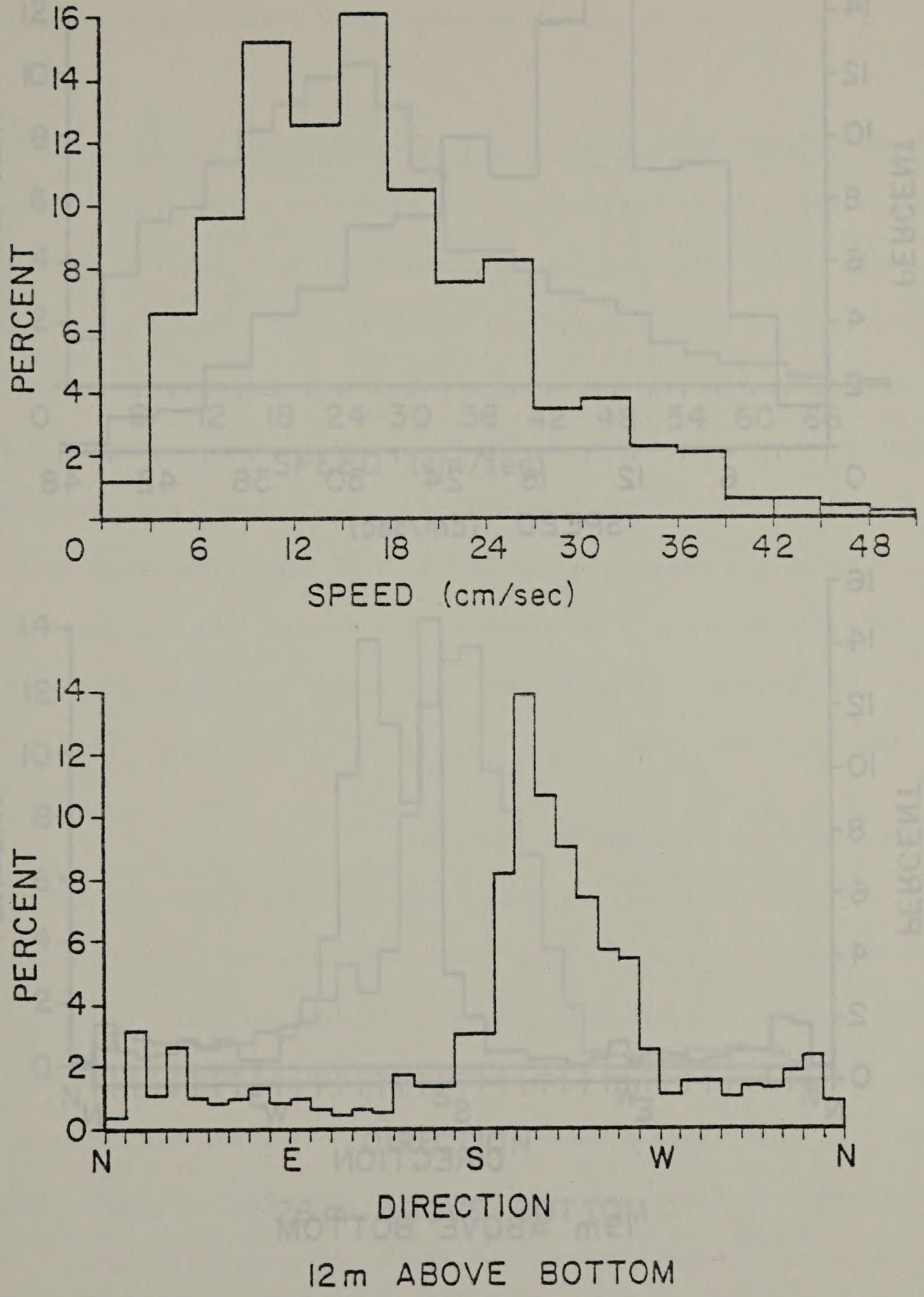

Figure 2.67 Histograms of Current Speed (3 cm/sec intervals) and Current Direction (10° intervals), 12 m Above Bottom, at Outer Station, 9 March through 26 April 1977.

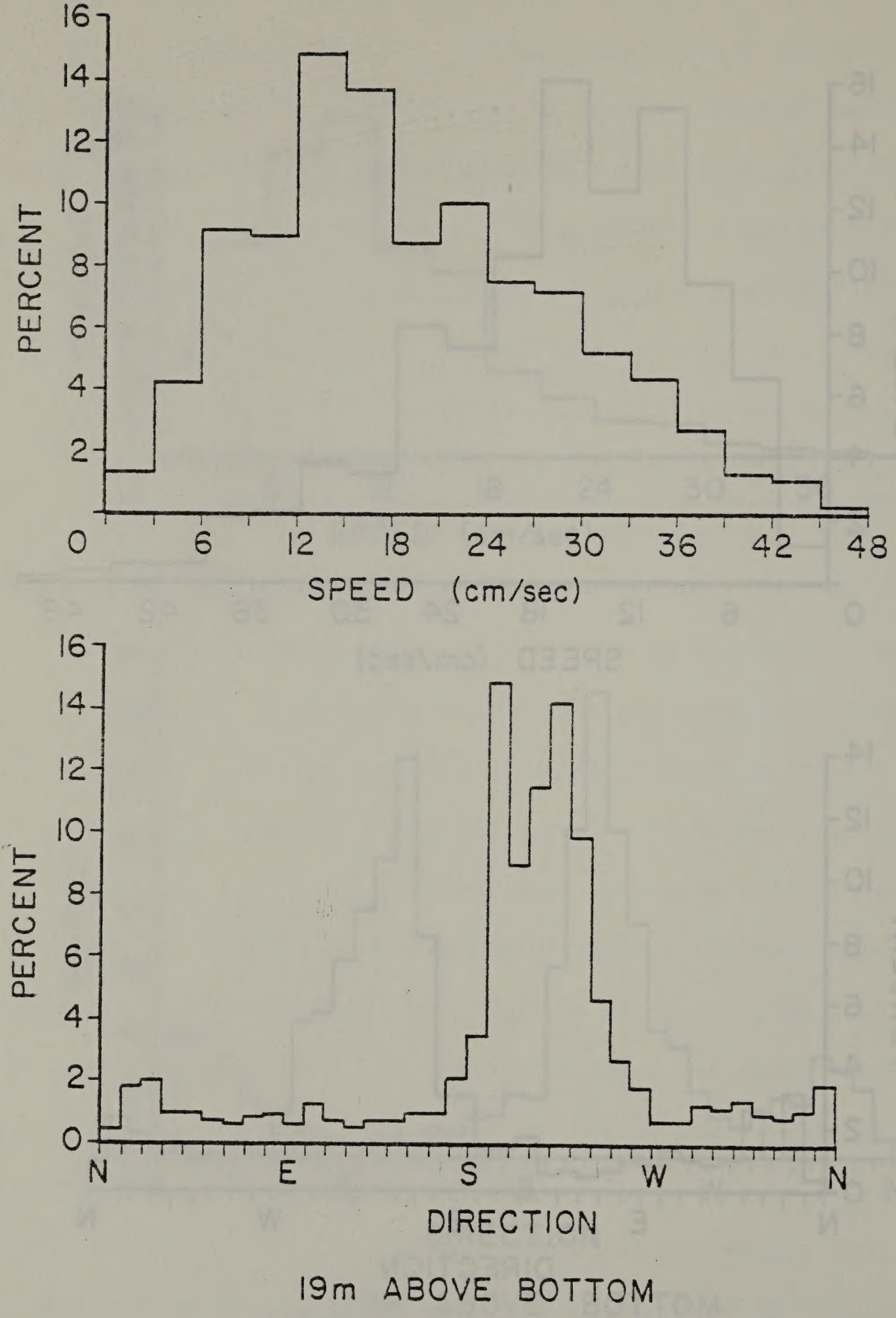

Figure 2.68 Histograms of Current Speed (3 cm/sec intervals) and Current Direction (10° intervals), 19 m Above Bottom, at Outer Station, 9 March through 26 April 1977.

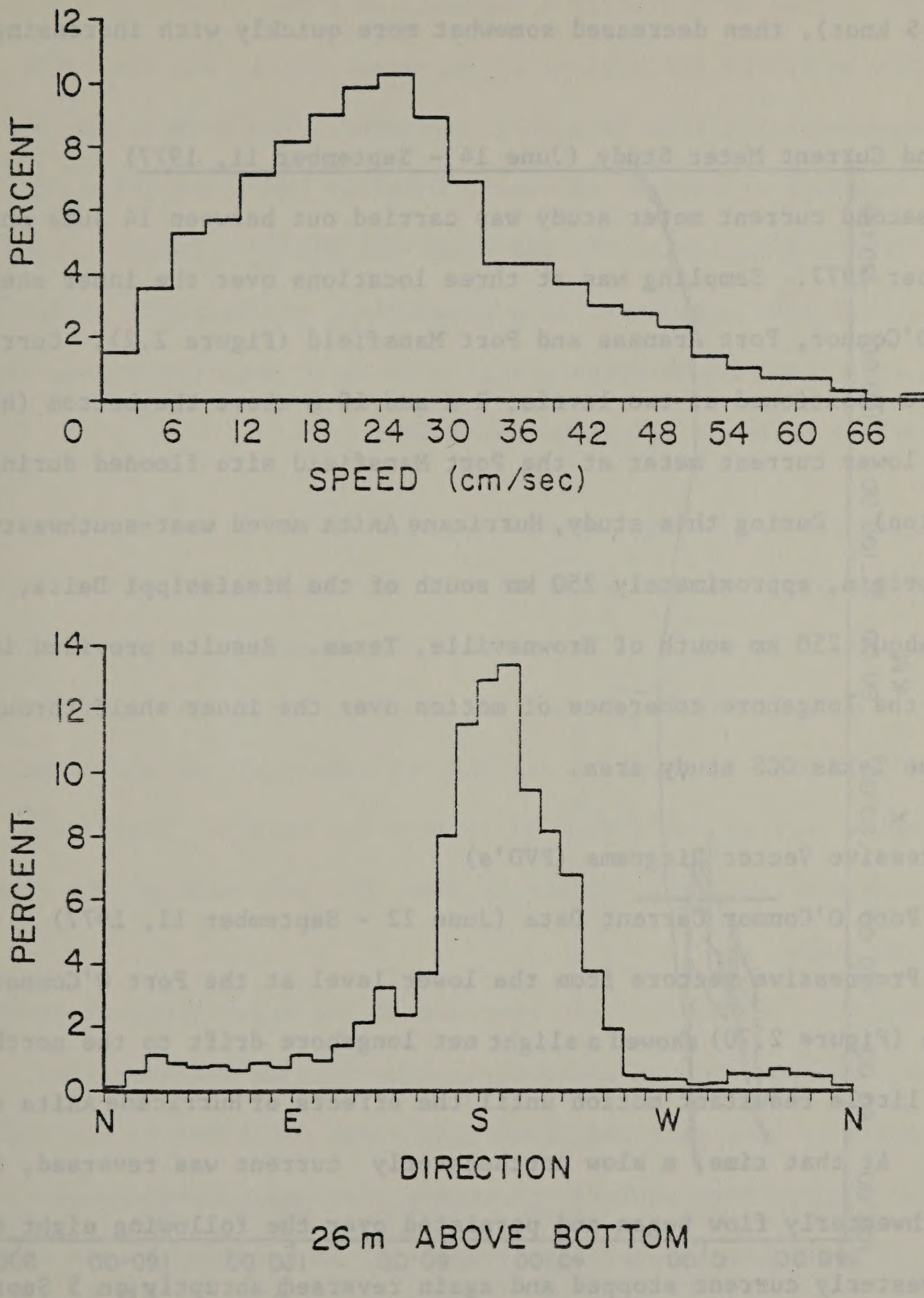

Figure 2.69 Histograms of Current Speed (3 cm/sec intervals) and Current Direction (10° intervals), 26 m Above Bottom, at Outer Station, 9 March through 26 April 1977.

where in the histogram, with some indication of higher values in the northeasterly quadrant than in the northwesterly quadrant. The speed histogram increased fairly uniformly to a maximum in the interval between 24 and 27 cm/sec (0.5 knot), then decreased somewhat more quickly with increasing speed.

Second Current Meter Study (June 14 - September 11, 1977)

The second current meter study was carried out between 14 June and 11 September 1977. Sampling was at three locations over the inner shelf off Port O'Connor, Port Aransas and Port Mansfield (Figure 2.2). Current meters were positioned at two levels, 2 m and 10 m above the bottom (however, the lower current meter at the Port Mansfield site flooded during installation). During this study, Hurricane Anita moved west-southwestward from its origin, approximately 250 km south of the Mississippi Delta, to landfall about 250 km south of Brownsville, Texas. Results provided information on the longshore coherence of motion over the inner shelf through most of the Texas OCS study area.

Progressive Vector Diagrams (PVD's)

Port O'Connor Current Data (June 22 - September 11, 1977)

Progressive vectors from the lower level at the Port O'Connor study site (Figure 2.70) showed a slight net longshore drift to the northeast, but little resultant motion until the effects of Hurricane Anita on August 26. At that time, a slow northeasterly current was reversed, and rapid southwesterly flow began and persisted over the following eight days. The southwesterly current stopped and again reversed abruptly on 5 September, and the current began to backtrack slowly. Though the longshore displacement of water was substantial during this time interval, due primarily to the hurricane effects, the cross-shelf displacement never exceeded 19 km in an onshore direction, or 23 km in an offshore direction.

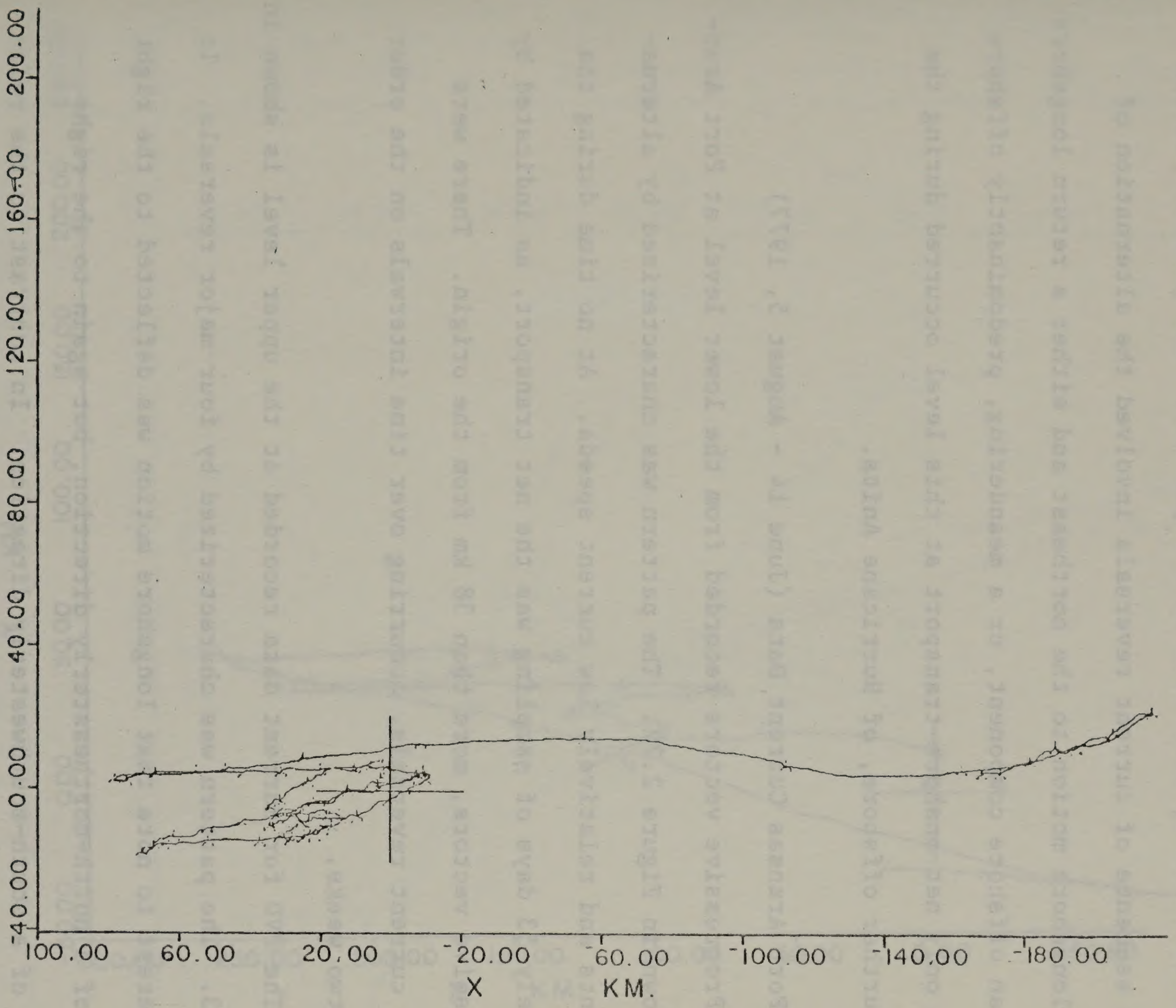

Figure 2.70 Progressive Vector Diagram of Currents Measured off Port O'Connor, 2 m Above the Bottom, Between 22 June and 11 September 1977. X Direction is Longshore, Positive Toward 0.62°; Y Direction is Positive Offshore.

The progressive vector diagram constructed from the data recorded at the upper level is shown in Figure 2.71. The stronger currents indicated at this level showed more clearly a sequence of seven reversals in the longshore motion before the arrival of Hurricane Anita, and the rapid return to northeasterly flow late on 5 September. The general pattern during the sequence of current reversals involved the alternation of bursts of longshore motion to the northeast and either a return longshore flow with an offshore component, or a meandering, predominantly offshore flow. The only net onshore transport at this level occurred during the passage, further offshore, of Hurricane Anita.

Port Aransas Current Data (June 14 - August 5, 1977)

Progressive vectors recorded from the lower level at Port Aransas are shown in Figure 2.72. The pattern was characterized by alternating currents and relatively low current speeds. At no time during the approximately 53 days of sampling was the net transport, as indicated by the progressive vectors, more than 38 km from the origin. There were four major current reversals, occurring over time intervals on the order of one to two weeks.

The PVD for current data recorded at the upper level is shown in Figure 2.73. The pattern was characterized by four major reversals. It was of interest to note that longshore motion was deflected to the right (seaward) of a north-northeasterly direction, but again to the right (landward) of a south-southwesterly direction. In contrast to the relatively minor longshore transport of water past the lower current meter, a cumulative displacement of approximately 144 km was indicated at the upper level toward the north-northeast. Over the same time interval, a net displacement of just over 60 km in an offshore direction was recorded.

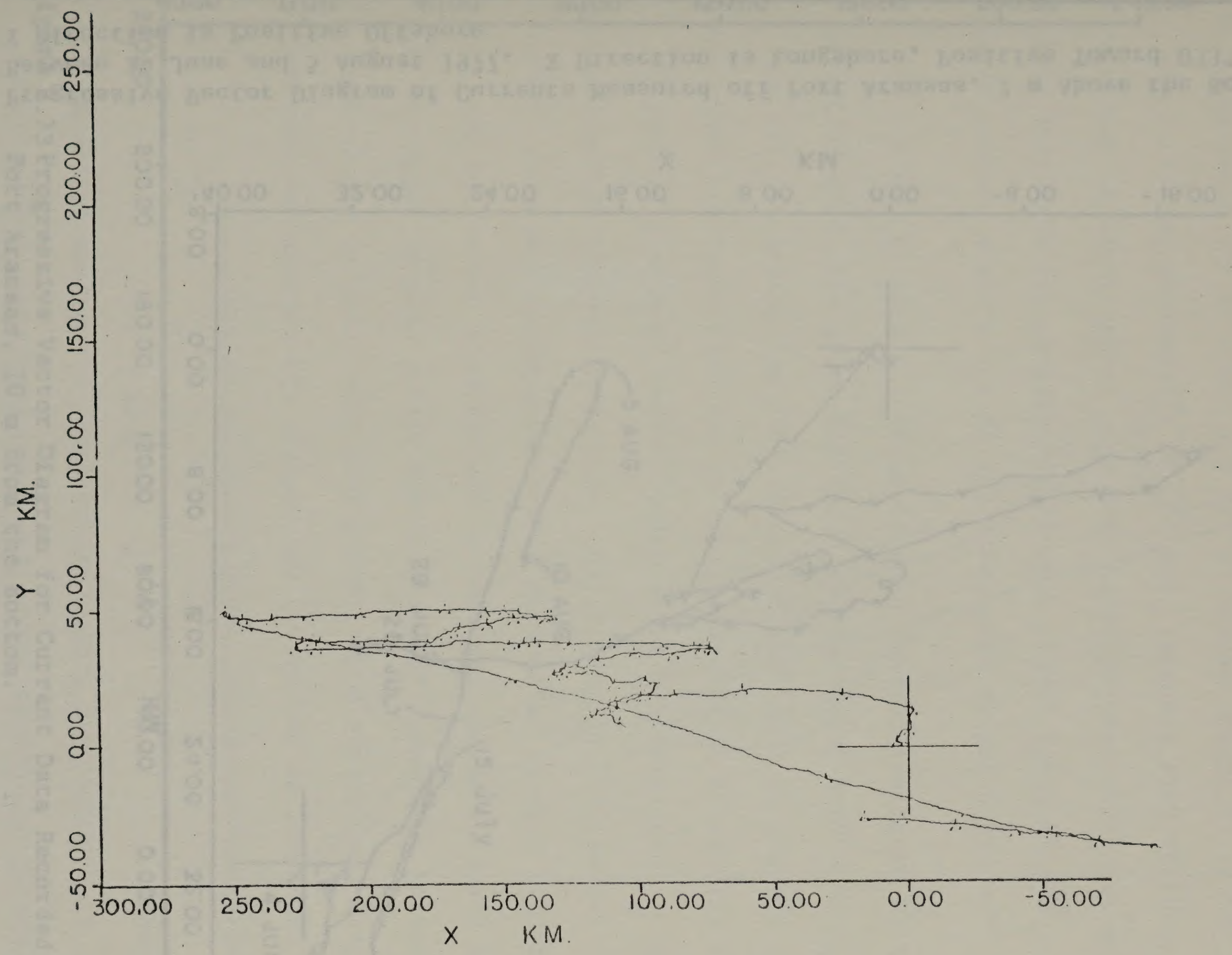

Figure 2.71 Progressive Vector Diagram of Currents Measured off Port O'Connor, 10 m Above the Bottom, Between 22 June and 11 September 1977. X Direction is Longshore, Positive Toward 062°; Y Direction is Positive Offshore.

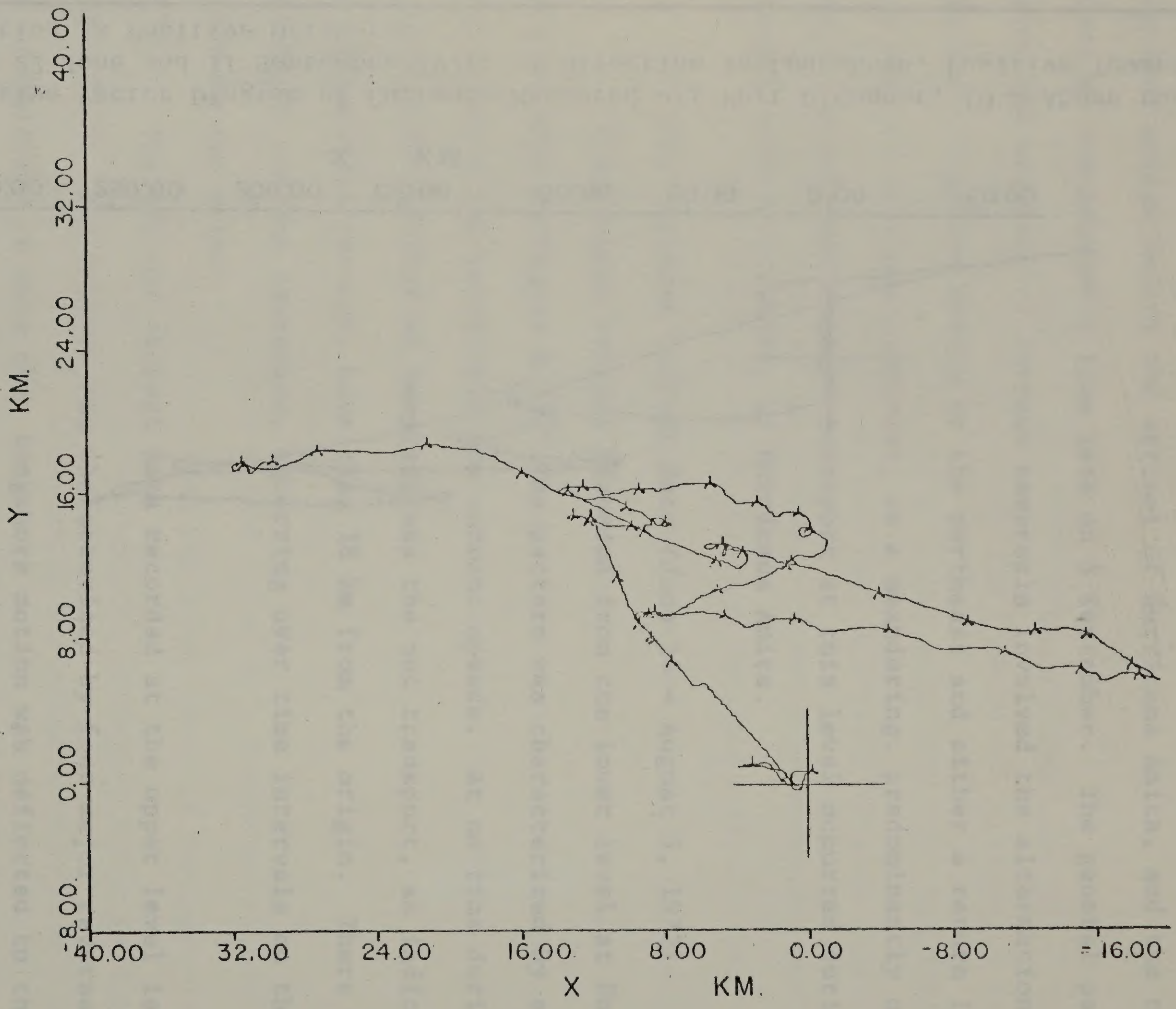

Figure 2.72 Progressive Vector Diagram of Currents Measured off Port Aransas, 2 m Above the Bottom, Between 14 June and 5 August 1977. X Direction is Longshore, Positive Toward 033°; Y Direction is Positive Offshore.

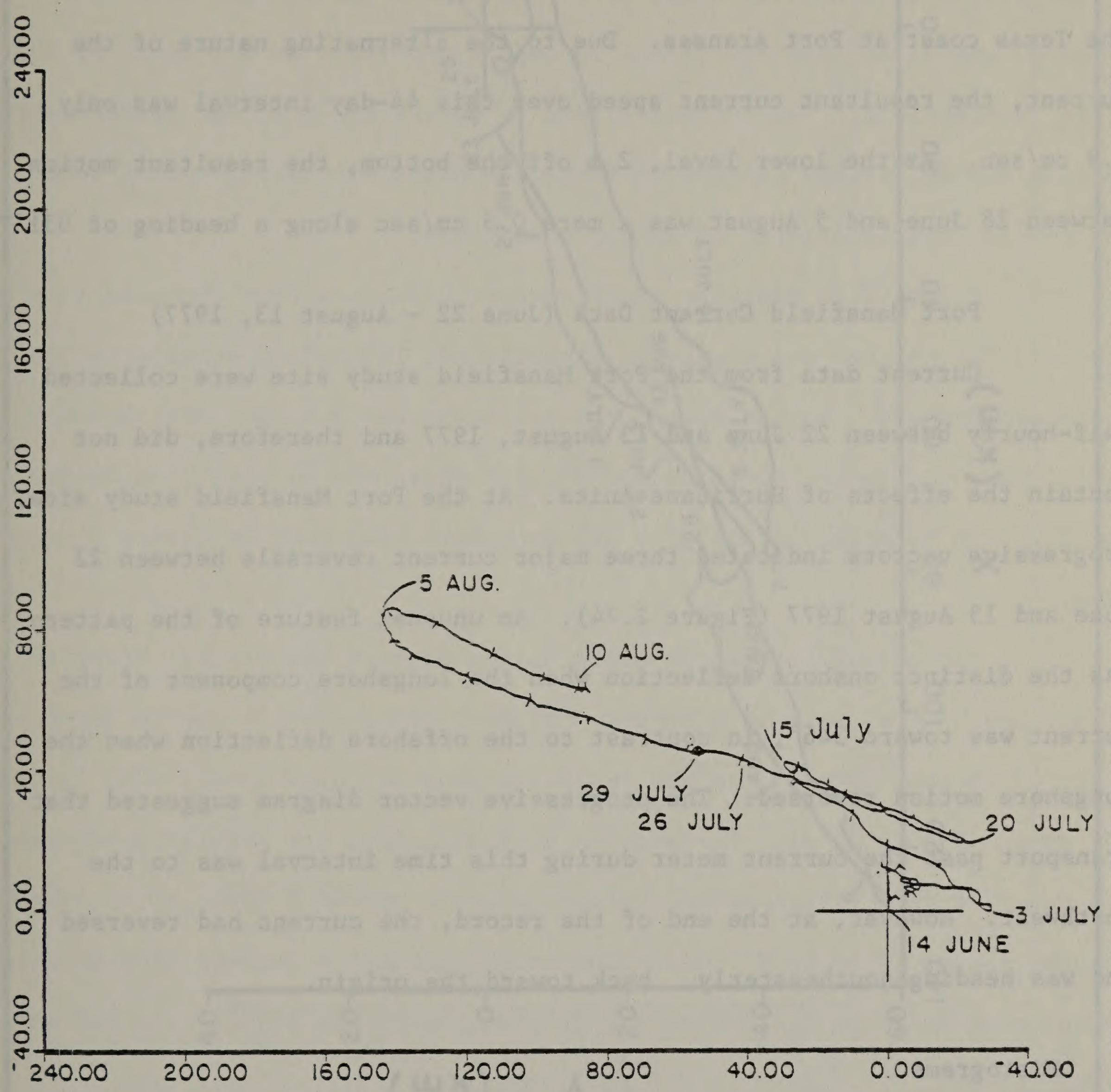

Figure 2.73 Progressive Vector Diagram for Current Data Recorded off Port Aransas, 10 m from the Bottom.

End points of the PVD's were used to determine the resultant speed and direction of the current past the study site. At the upper level, the resultant motion between 28 June and 10 August was along a heading of 065°T, or approximately 32° to the right of the orientation of the Texas coast at Port Aransas. Due to the alternating nature of the current, the resultant current speed over this 44-day interval was only 2.9 cm/sec. At the lower level, 2 m off the bottom, the resultant motion between 28 June and 5 August was a mere 0.5 cm/sec along a heading of 051°T.

Port Mansfield Current Data (June 22 - August 13, 1977)

Current data from the Port Mansfield study site were collected half-hourly between 22 June and 13 August, 1977 and therefore, did not contain the effects of Hurricane Anita. At the Port Mansfield study site, progressive vectors indicated three major current reversals between 22 June and 13 August 1977 (Figure 2.74). An unusual feature of the pattern was the distinct onshore deflection when the longshore component of the current was toward 346°, in contrast to the offshore deflection when the longshore motion reversed. The progressive vector diagram suggested that transport past the current meter during this time interval was to the northwest. However, at the end of the record, the current had reversed and was heading southeasterly, back toward the origin.

Histograms

Again, histograms were presented for each of the time series as a means of summarizing information on speed and direction distributions individually. Results showed the alternating longshore current characteristic of the summer months along the Texas coast, and the generally low current speeds.

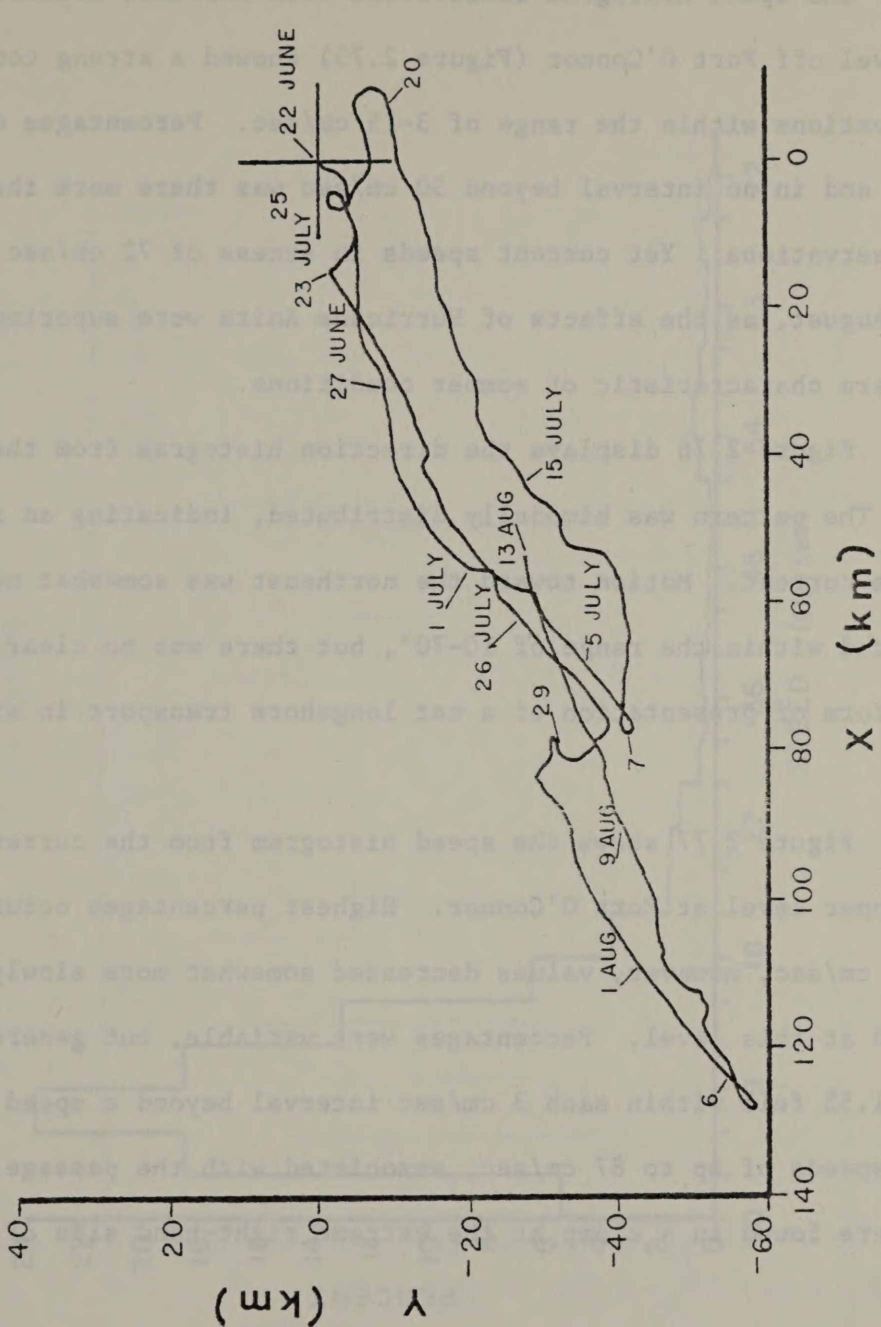

Figure 2.74 Progressive Vector Diagram of Currents Measured off Port Mansfield, 10 m Above Bottom, 22 June - 13 August 1977. X-direction is Longshore, Positive toward 346°; Y-direction is Positive Offshore.

Port O'Connor Current Data

The speed histogram constructed from currents measured at the lower level off Port O'Connor (Figure 2.75) showed a strong concentration of observations within the range of 3-15 cm/sec. Percentages dropped off quickly, and in no interval beyond 30 cm/sec was there more than 1% of the total observations. Yet current speeds in excess of 72 cm/sec were recorded in late August, as the effects of Hurricane Anita were superimposed onto the pattern characteristic of summer conditions.

Figure 2.76 displays the direction histogram from the same time series. The pattern was bimodally distributed, indicating an alternating longshore current. Motion toward the northeast was somewhat more broadly distributed within the range of 10-70°, but there was no clear indication in this form of presentation of a net longshore transport in either direction.

Figure 2.77 shows the speed histogram from the current recorded at the upper level at Port O'Connor. Highest percentages occurred between 3 and 15 cm/sec, however, values decreased somewhat more slowly with increasing speed at this level. Percentages were variable, but generally between 0.5 and 1.5% fell within each 3 cm/sec interval beyond a speed of 45 cm/sec. Highest speeds of up to 87 cm/sec, associated with the passage of Hurricane Anita, were found in a clump at the extreme right-hand side of the histogram.

The direction histogram from the upper level (Figure 2.78) indicated a bimodal pattern, with a suggestion of a preference for northeasterly motion and a somewhat broader distribution of directions for currents to the southwest.

Port Aransas Current Data

The speed histogram from the lower level (Figure 2.79) indicated

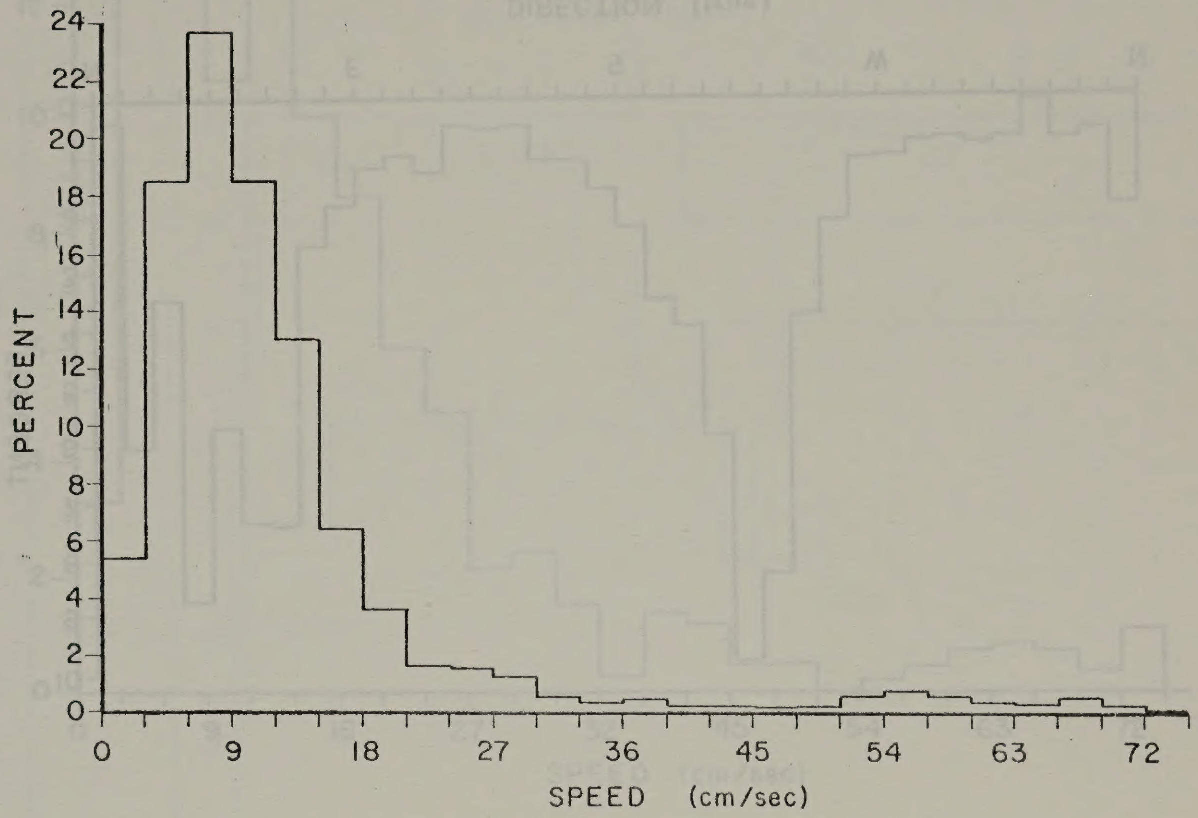

Figure 2.75 Histogram of Current Speeds, 2 m Above the Bottom, off Port O'Connor, 22 June through 11 September 1977.

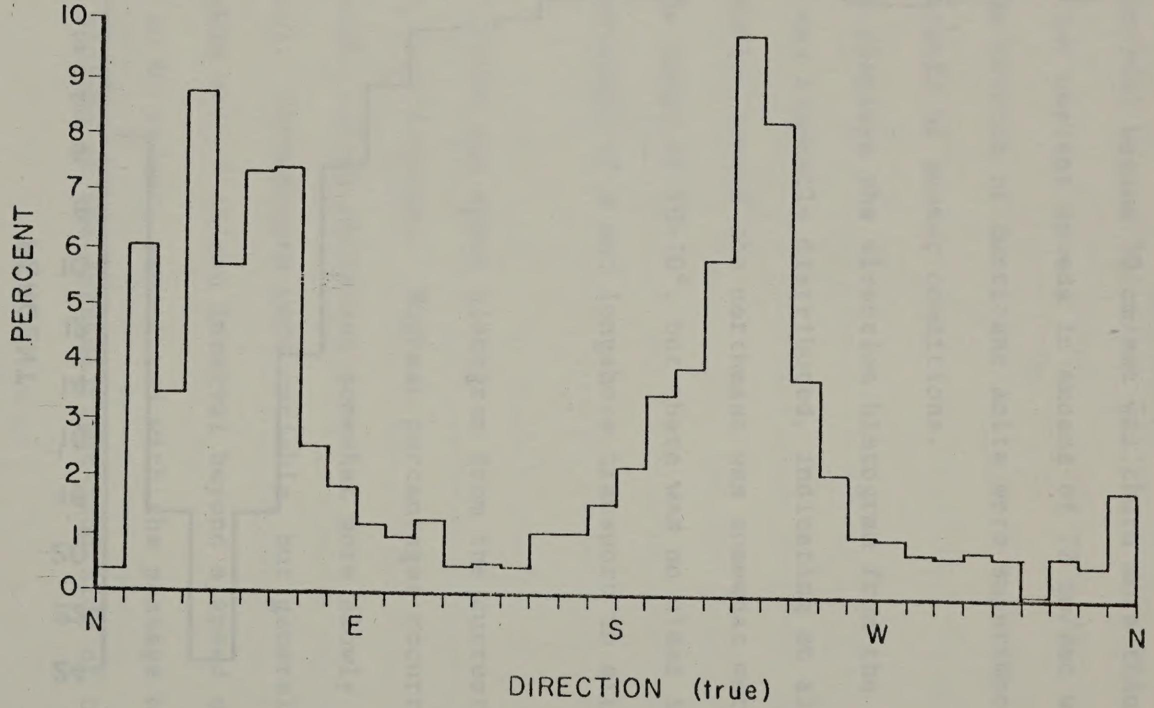

Figure 2.76 Histogram of Current Direction, 2 m Above the Bottom, off Port O'Connor, 22 June through 11 September 1977.

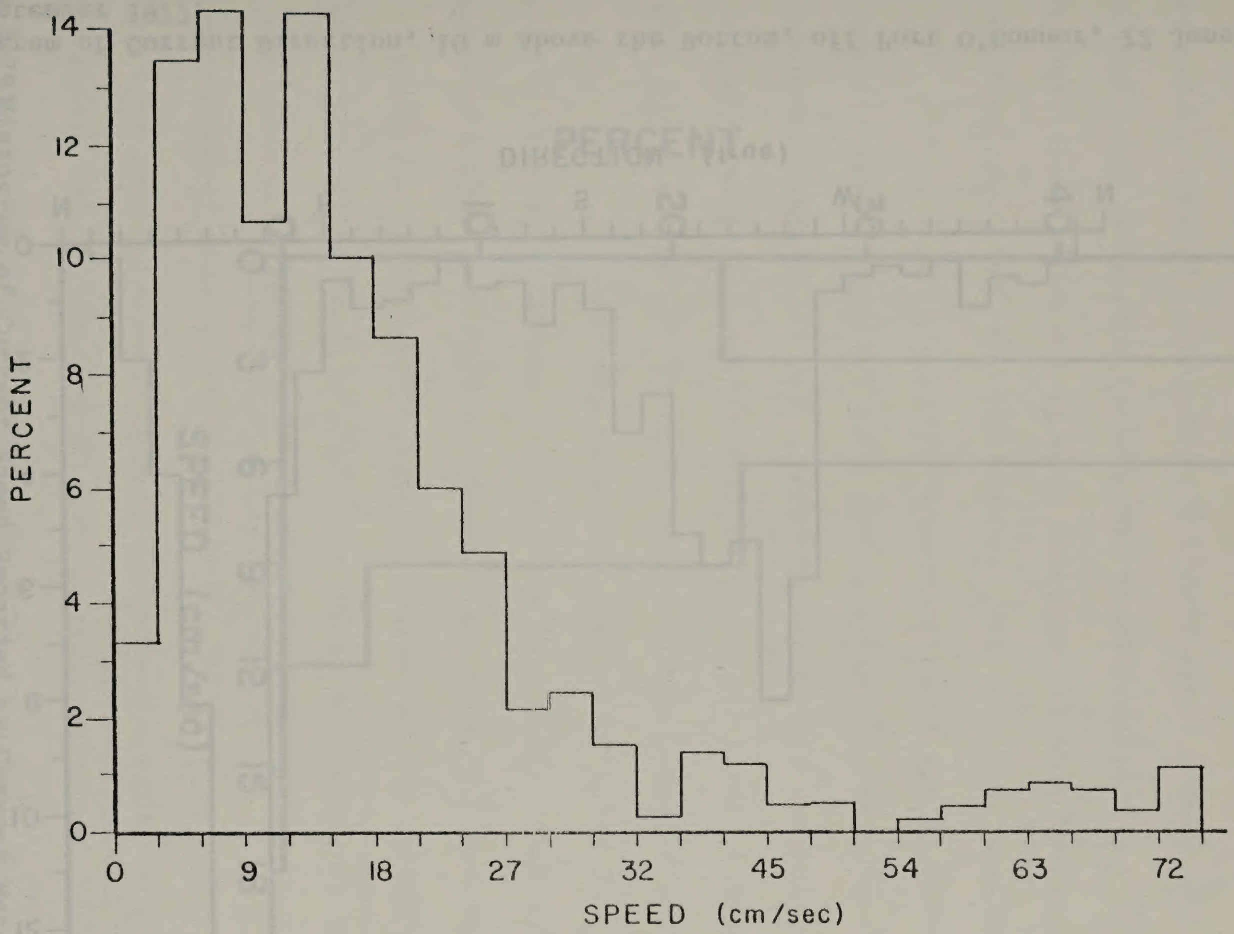

Figure 2.77 Histogram of Current Speed, 10 m Above the Bottom, Off Port O'Connor, 22 June through 11 September 1977.

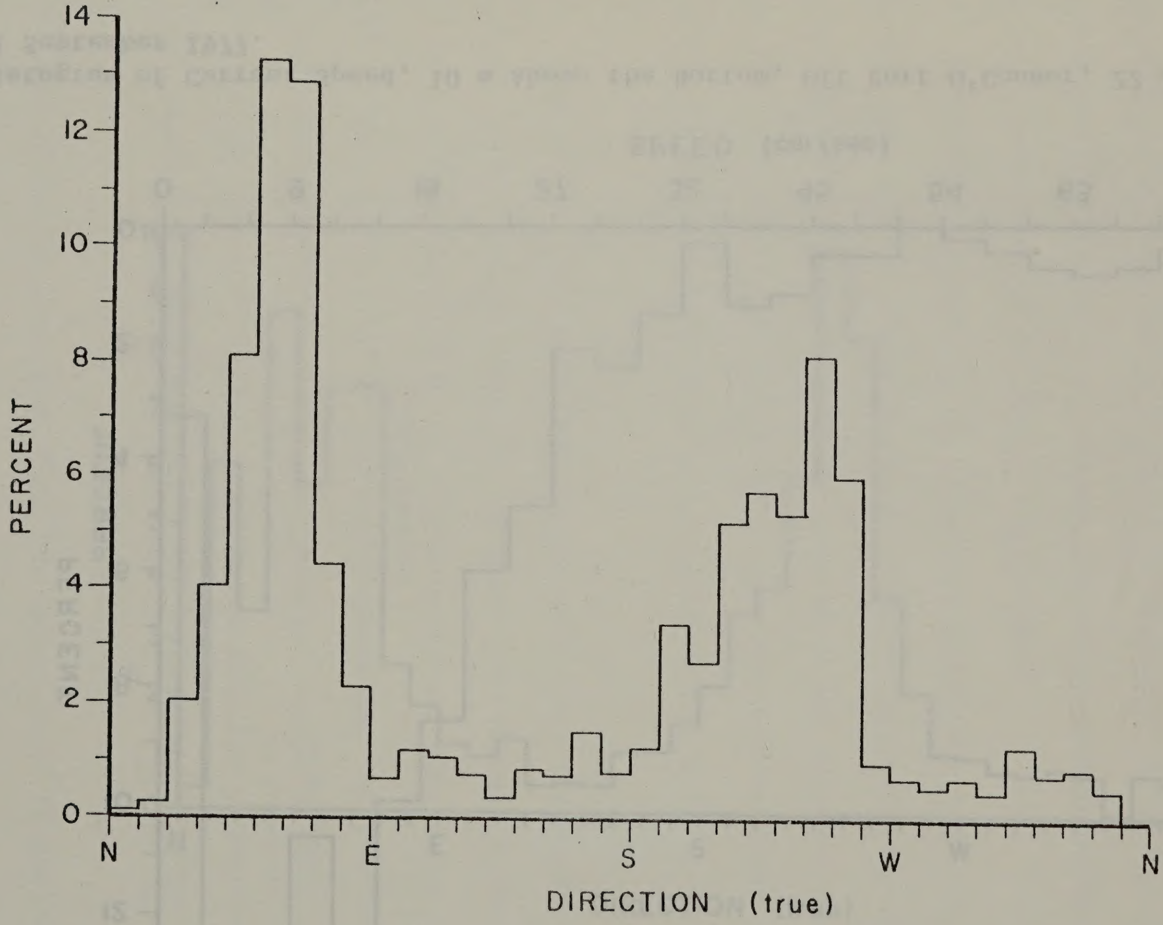

Figure 2.78 Histogram of Current Direction, 10 m Above the Bottom, off Port O'Connor, 22 June through 11 September 1977.

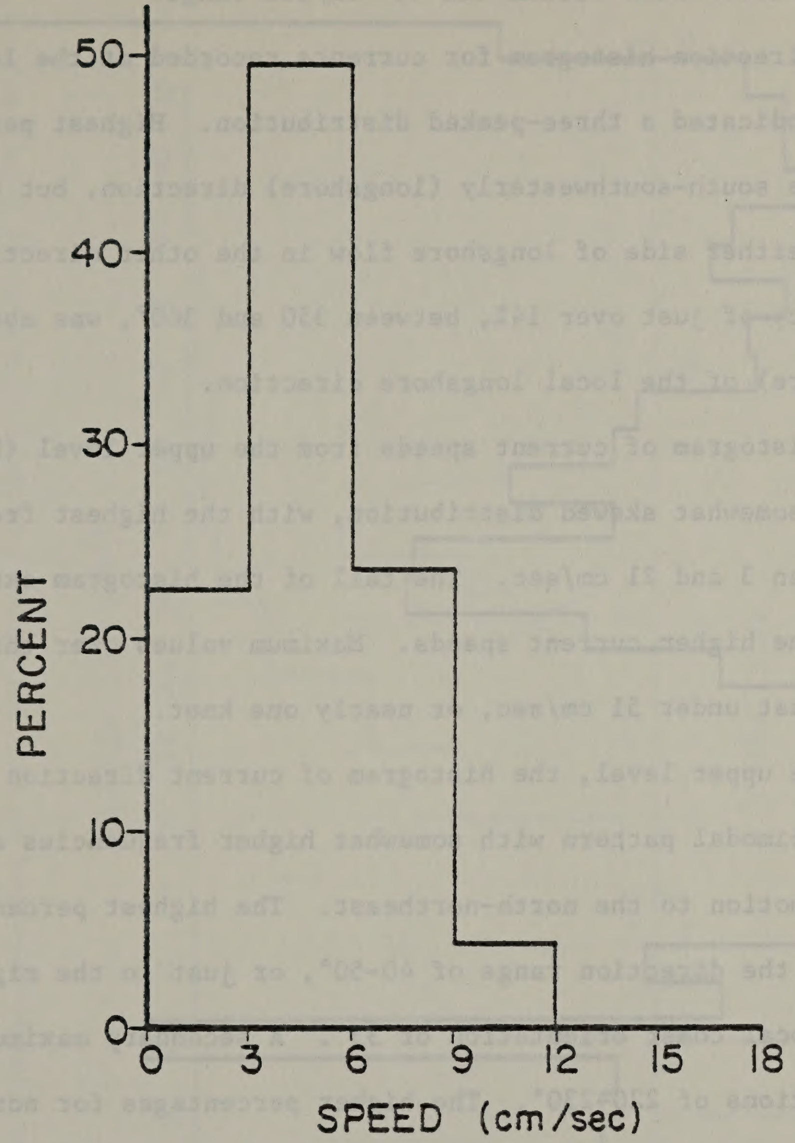

Figure 2.79 Histogram of Current Speed Recorded by Current Meter off Port Aransas, 2 m off Bottom, in 18 m of Water, 14 June through 5 August 1977.

a surprising concentration of currents in the low-speed range. No current speeds in excess of 12 cm/sec were measured over this time interval. Highest frequencies were found within the 36 cm/sec range.

The direction histogram for currents recorded at the lower level (Figure 2.80) indicated a three-peaked distribution. Highest percentages appeared along a south-southwesterly (longshore) direction, but there were two maxima on either side of longshore flow in the other direction. The highest frequency of just over 14%, between 350 and 360°, was about 40° to the left (onshore) of the local longshore direction.

The histogram of current speeds from the upper level (Figure 2.81) showed a somewhat skewed distribution, with the highest frequency generally between 3 and 21 cm/sec. The tail of the histogram extended well out into the higher current speeds. Maximum values over this time interval were just under 51 cm/sec, or nearly one knot.

At the upper level, the histogram of current direction (Figure 2.82) showed a bimodal pattern with somewhat higher frequencies associated with longshore motion to the north-northeast. The highest percentage occurred within the direction range of 40-50°, or just to the right (offshore) of the local coast orientation of 33°. A secondary maximum was found for directions of 220-230°. The higher percentages for north-northeasterly flow were consistent with the general pattern of longshore motion to the north along the Texas coast in the summer months. The bimodal distribution of current direction, however, suggested that the concept of a unidirectional flow, and the resultant motion calculations, were an oversimplification.

Port Mansfield Current Data

The histogram of current speeds recorded 10 m above the bottom (Figure 2.83) showed highest percentages in speed intervals between 3 and

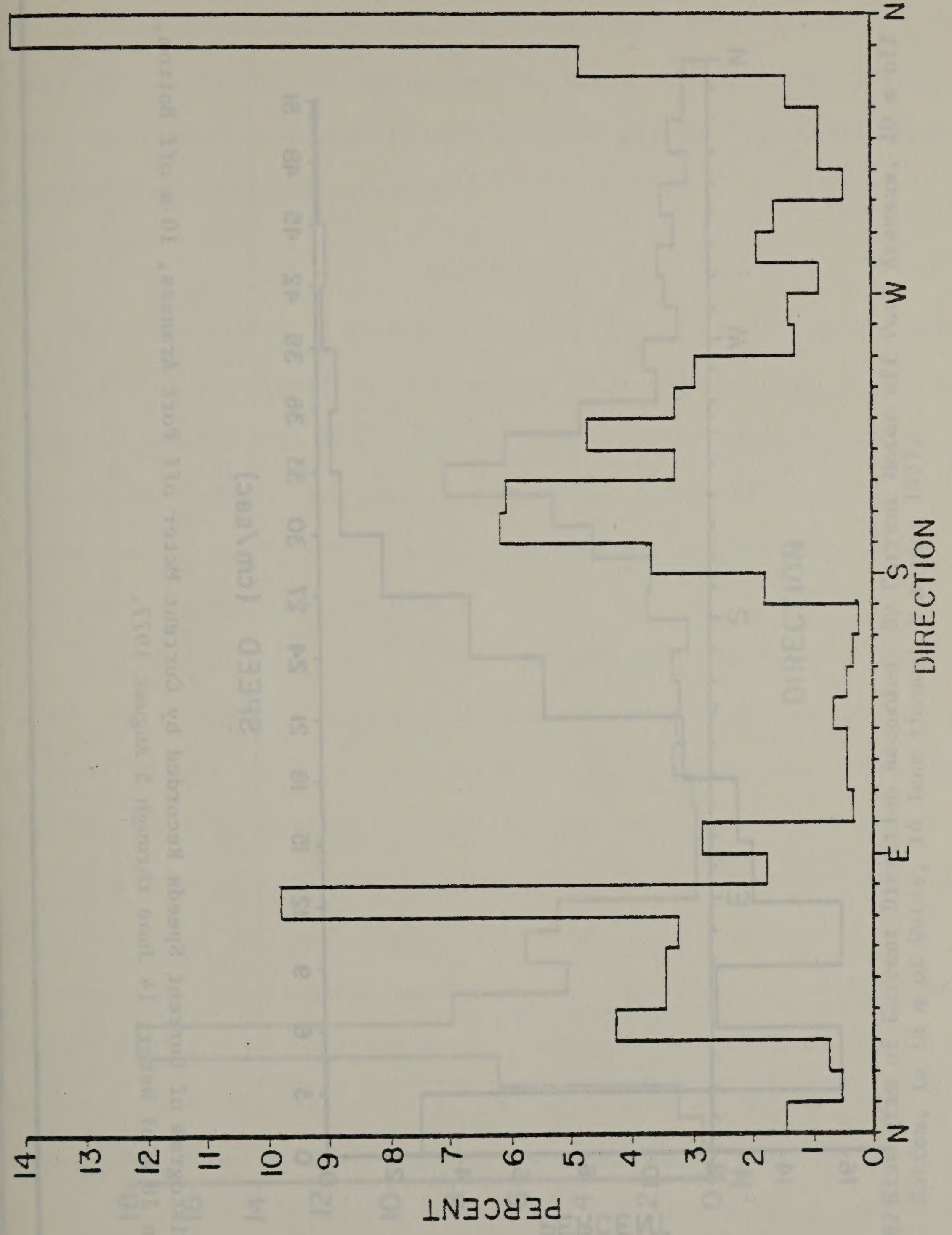

Figure 2.80 Histogram of Current Direction Recorded by Current Meter off Port Aransas, 2 m off Bottom, in 18 m of Water, 14 June through 5 August 1977.

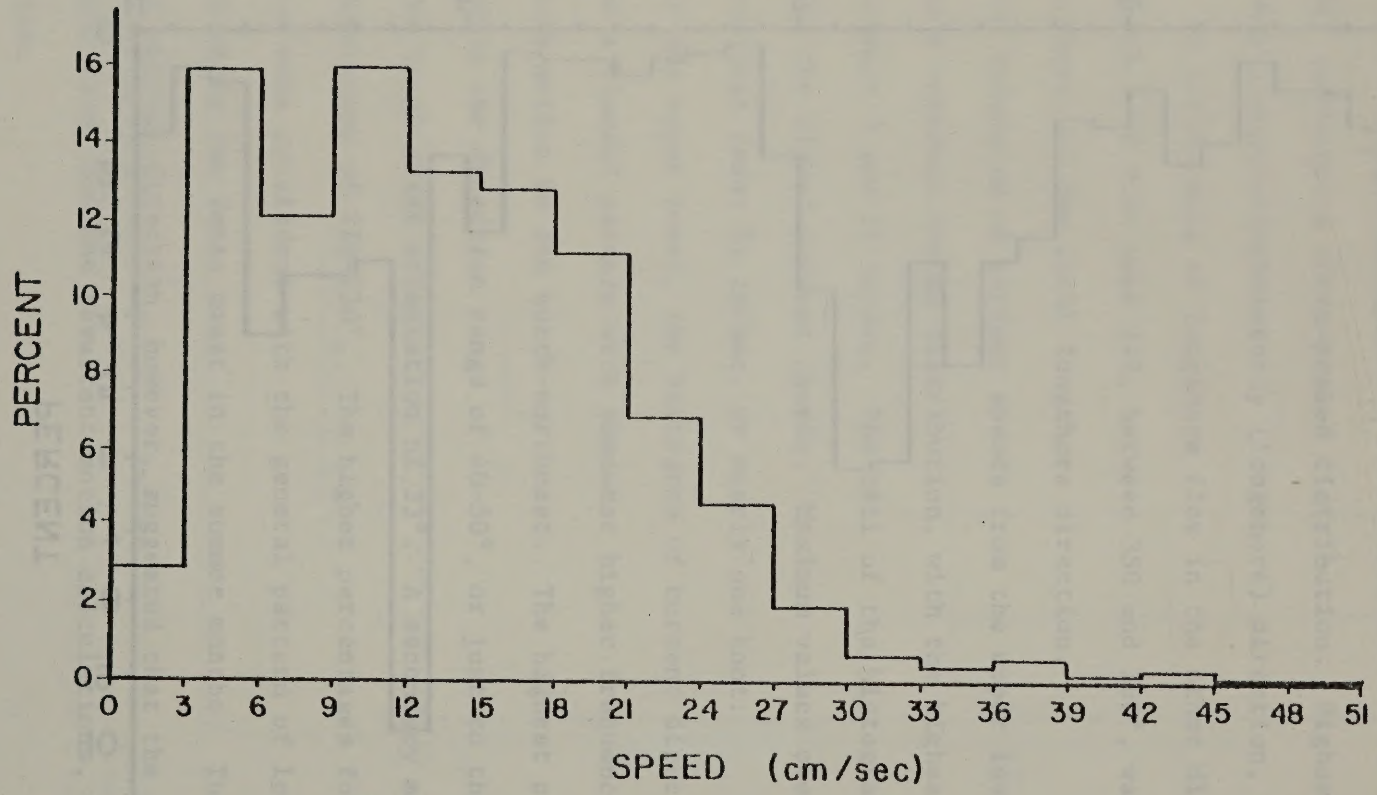

Figure 2.81 Histogram of Current Speeds Recorded by Current Meter off Port Aransas, 10 m off Bottom, in 18 m of Water, 14 June through 5 August 1977.

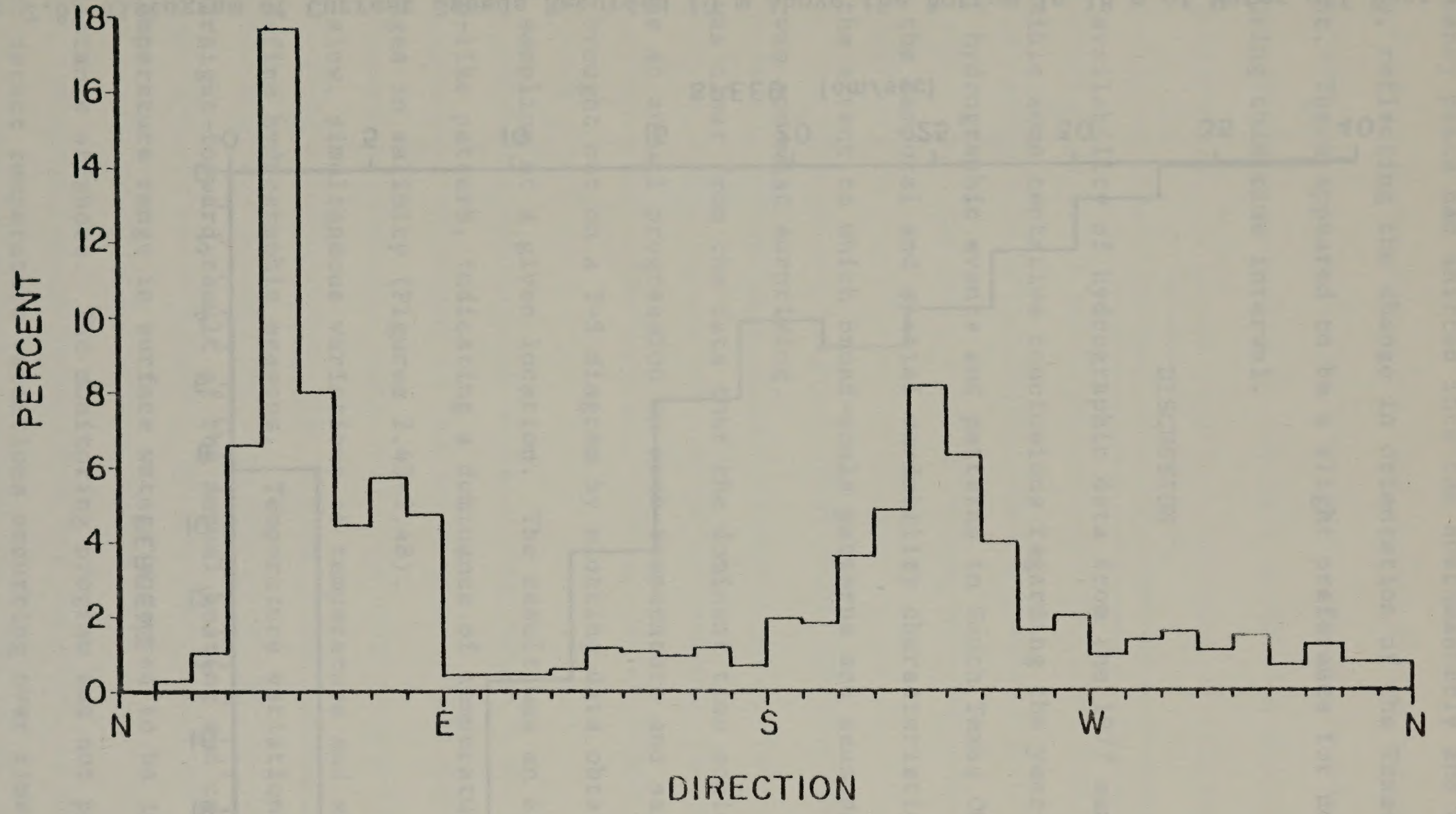

Figure 2.82 Histogram of Current Direction Recorded By Current Meter off Port Aransas, 10 m off Bottom, in 18 m of Water, 14 June through 5 August 1977.

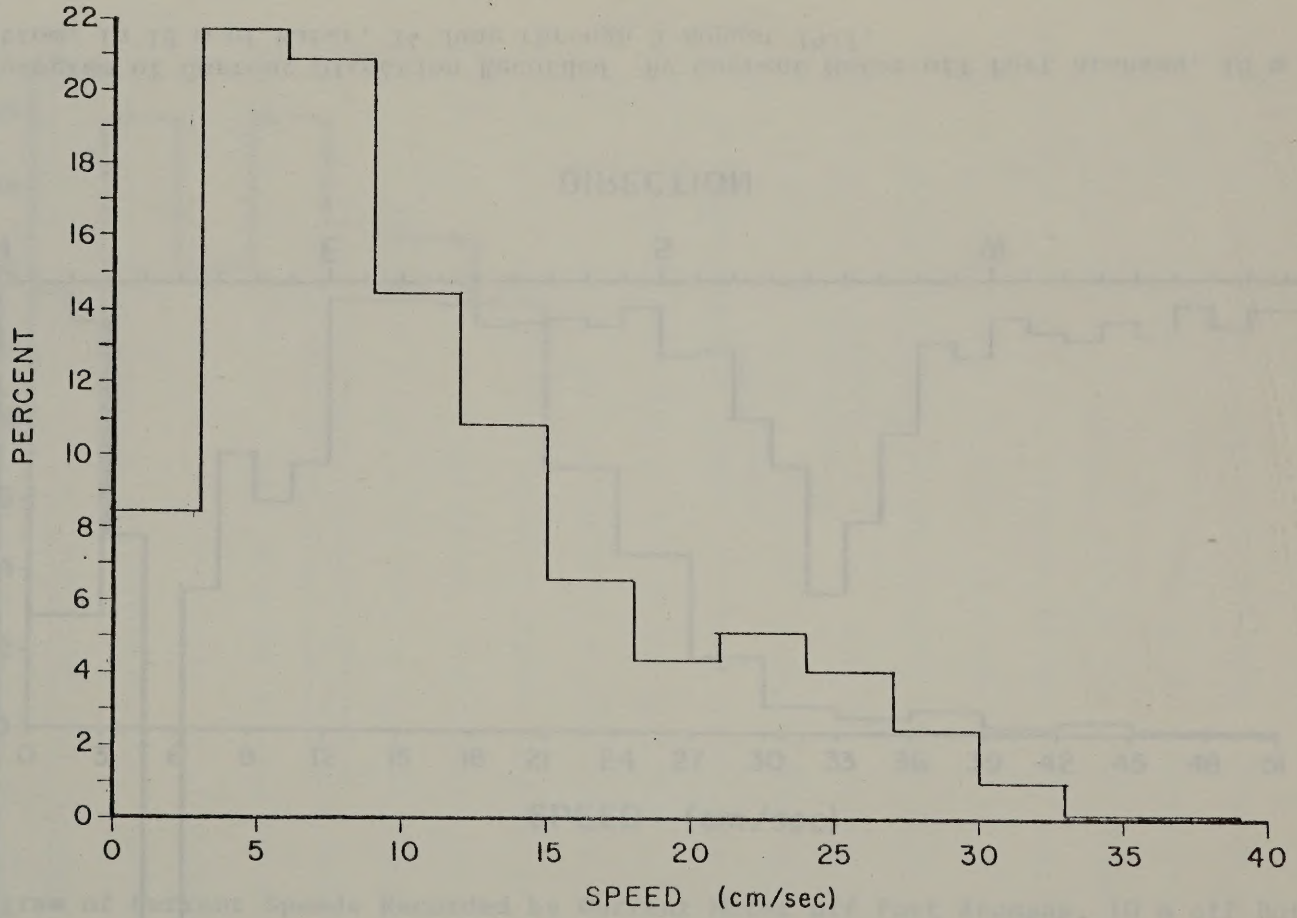

Figure 2.83 Histogram of Current Speeds Recorded 10 m Above the Bottom in 18 m of Water off Port Mansfield 22 June through 13 August 1977

9 cm/sec. All current speeds were less than 39 cm/sec. The histogram of current directions from Port Mansfield (Figure 2.84) was bimodal, however, the frequency peaks had shifted into the southeasterly and northwesterly quadrants, reflecting the change in orientation of the Texas coastline at this point. There appeared to be a slight preference for northwesterly motion during this time interval.

DISCUSSION

The availability of hydrographic data from the 1977 sampling program made possible some tentative conclusions regarding the year-to-year repetition of hydrographic events and patterns in South Texas OCS waters. Considering the temporal and spatial variability characteristics of shelf waters, the extent to which broad-scale patterns and seasonal events were repeated was somewhat surprising.

It was clear from the data that the dominant time scale in Texas shelf waters was an annual progression in both temperature and salinity. This was best brought out on a T-S diagram by plotting data obtained from repeated sampling at a given location. The result was an elongated, clockwise loop-like pattern, indicating a dominance of temperature variations over changes in salinity (Figures 2.43-2.48).

The slow, simultaneous variations of temperature and salinity were used to define hydrographic seasons. Temperature variations were a conceptually straight-forward result of the annual heating and cooling. The annual temperature range in surface waters appeared to be inversely related to the distance offshore. The monitoring program was not particularly well suited to detect temperature variations occurring over time scales of less than several weeks. Given the magnitude of the annual temperature curve, however, it was probable that diurnal temperature variations and those

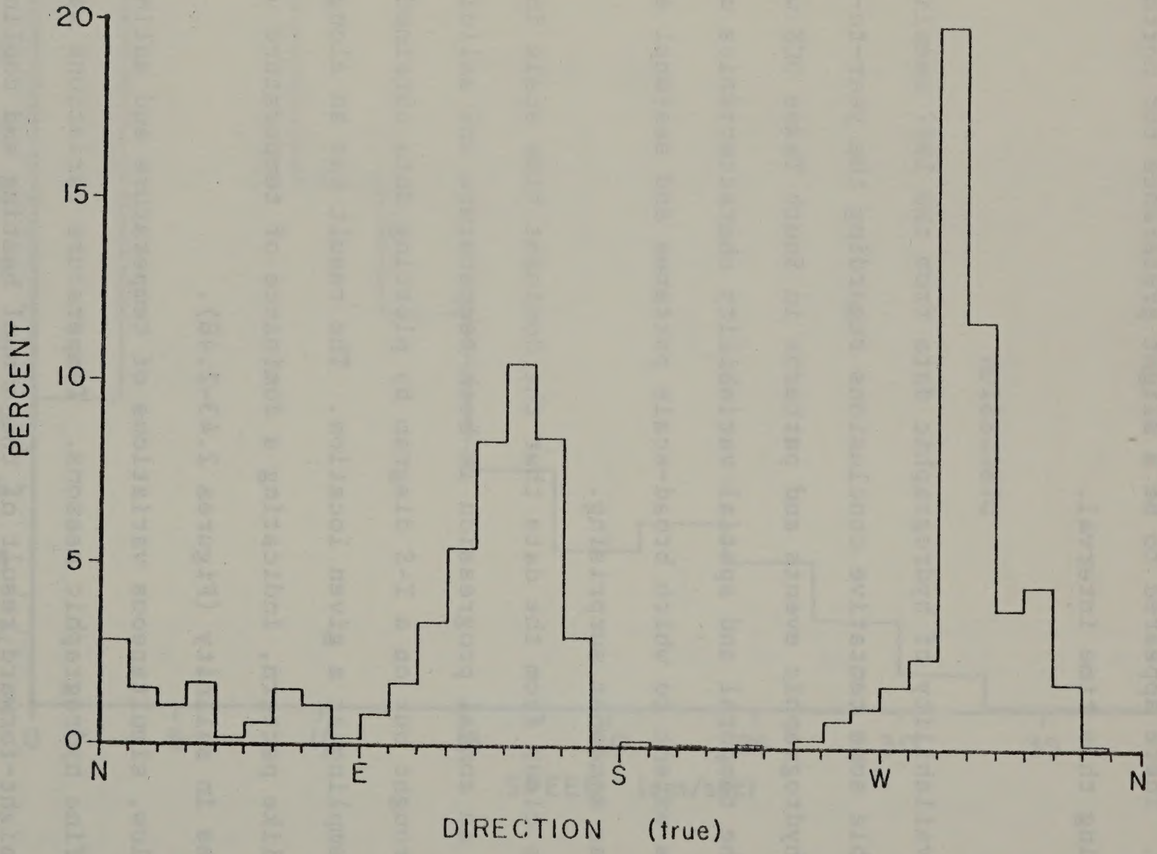

Figure 2.84 Histogram of Current Directions Recorded 10 m Above the Bottom in 18 m of Water off Port Mansfield, 22 June through 13 August.

associated with advective processes were minor perturbations on the annual pattern.

Highest late summer temperatures in surface waters have been shown to be nearly uniform across the shelf, and indeed across the entire Gulf of Mexico (Rivas, 1968). Thus, the local magnitude of the annual temperature curve was determined primarily by the late winter low temperature reached at a given location. This was largely a function of the heat content of the water column, which, in turn, was directly related to the water depth. A second, though probably relatively minor effect, was the fact that the cold air following a frontal passage began to warm as it moved out over shelf waters. Thus, the most intense cooling probably occurred over the inner shelf.

At sub-surface levels, highest late-summer temperatures decreased with increasing depth. This was especially true below the layer of wind mixing (upper 20-30 m), which capped the top of the seasonal thermocline. Still, an annual progression of warming and cooling was clearly apparent over the inner and middle shelf.

As noted in the description accompanying Figure 2.48, however, near-bottom temperature variations at the outer edge of the shelf, at the top of the permanent thermocline, were occurring over time scales considerably shorter than the annual heating and cooling noted elsewhere. There, advective processes and/or perhaps vertical migrations in the top of the permanent thermocline, produced temporal changes that could not be defined by the approximately monthly monitoring during 1977.

Where the annual temperature curve was apparent, it could be used to define hydrographic seasons in a general way. The winter months were characterized by surface cooling and the development and slow thickening of an isothermal surface layer. This extended through the entire water column over the inner and middle continental shelf. Over the outer shelf, the isothermal layer reached a depth of 70-80 m. In some cases, a slight reverse thermocline developed where vertical variations in salinity were great enough to preserve the stability of the water column. An important distinguishing feature of the winter months was the cross-shelf variation of surface temperature. Figures 2.3, 2.7 and 2.9, for example, showed a cross-shelf gradient of approximately 8°/60 km over the inner and middle shelf.

Spring, in a hydrographic sense, was best defined by the transient decrease in surface layer salinity associated with the spring runoff. For the waters of the South Texas coast, this time period extended from approximately late April into early June. The entire Texas shelf exhibited noticeably lowered salinities (Figure 2.23), however, the strongest haloclines were found, as might be expected, over the inner and middle shelf. Strong cross-shelf surface temperature gradients almost disappeared (Figure 2.23), as the shallower waters of the inner shelf responded more quickly to seasonal warming.

The summer season was also defined in terms of the temperature structure of shelf waters. The characteristic feature at this time of year was the strong thermal stratification through the seasonal thermocline. Figure 2.27, for example, shows a nearly isothermal wave-mixed layer extending through the upper 35-40 m and capping the seasonal thermocline. Highest mid-summer surface temperatures reached 29-30° in Texas waters. A notable feature of the temperature field was the continuation of the nearly isothermal surface layer (Figure 2.37). The September hydrographic data (ironically collected on what was called the "Fall Seasonal Cruise") showed some indication of lower salinities over the inner shelf along Transects I and II (Figure 2.38), however, for the most part, values fell within a few tenths of 36 ppt.

The fall season began with the first cold front during the latter part of the year. This could have occurred anywhere from mid-September to early October. The movement of relatively cool air out over the shelf produced a slight decrease in surface water temperatures from the annual maximum, and started to erode the seasonal thermocline. The waters of the inner shelf showed a more rapid cooling, and by the end of the year the cross-shelf gradient appeared again, superimposed onto a net cooling on the order of 6-8°C.

It should be pointed out that there was no *a priori* reason for selecting four hydrographic seasons. This came out of an inspection of the data itself. Based on the temperature data alone, one might choose to define only two seasons; a winter, with a generally unstratified water column, followed by a summer characterized by strong thermal stratification. On the other hand, the salinity data alone suggested a spring season, characterized by lower salinities over the inner half of the shelf, followed by the remainder of the year in which values varied little from 36 ppt.

It was only by integrating the T-S data that one could identify an annual progression with four segments. Even then, there was some question regarding the distinction between fall and winter. The varying degree to which spatial and/or temporal variations stood out in the annual progression made it possible to identify almost any number of time periods, or "seasons", depending on what one wished to recognize or ignore. The features discussed herein were those which appeared to recur annually; deep convective overturning, especially in the late winter months; the brackish water plume in late spring; summer heating and stratification through mid-September; and fall cooling, starting with the first cold fronts. Perhaps the important point was that the selection of discrete hydrographic seasons were based upon an accumulation of data, rather than just mirroring the calendar seasons.

The 1977 data clearly showed the dominance of the annual time scale over factors associated with meteorologically driven advection or energy exchanges, which occurred over periods on the order of a week or two. The sampling program was not intended to monitor these higher frequency variations, but the continuity apparent from one approximately monthly sample to the next suggested that little of importance had occurred simultaneously to disrupt the slowly unfolding annual pattern.

The availability of current data from two time periods during the year made possible some preliminary generalizations regarding the annual variation in circulation patterns in Texas shelf waters. The most obvious difference between the data coming out of these two field experiments was in the computed net transport. During the March-April study, transport past most of the current meters was longshore to the south-southwest; the two notable exceptions were at the lower level at the inner station and at the upper most level at the outer station, where a strong offshore deflection was recorded (Figures 2.58 and 2.63). This was most probably a direct response to surface windstress, which, in turn, had a longshore component almost continuously to the south-southwest.

In sharp contrast to this, the data from the summer months showed little preference for longshore motion in either direction. The twin-peaked current direction histograms (*e.g.*, Figures 2.76, 2.78, 2.80, 2.82 and 2.84) reflected the effects of more nearly directly onshore winds with a slowly reversing longshore component. The annual pattern that emerged was one of transport to the south-southwest occurring in distinct spurts. For the central Texas coast, at least, the annual sequence appeared to include a strong longshore transport from late fall through early summer (approximately early November through early June) with little net motion the rest of the year. At any given time there was generally a significant current; however, the net long-term effect was a north-northeasterly to south-southwesterly transport along the central Texas coast.

Based on all of the above, the most important physical processes for controlling the hydrography of the South Texas OCS study area may be listed chronologically and, at the same time, in the approximate order of decreasing importance. Cold fronts moving off the central Texas coast are responsible for intense surface cooling, for a deep convective overturning of the water column, and for providing the wind-driven push responsible for the annual net transport to the south-southwest. The spring runoff, entering the Gulf of Mexico primarily through the mouth of the Mississippi River, gets caught up in the last of the westerly and southwesterly circulation along the northern rim of the Gulf, and arrives as a brackish water near-surface plume in late spring. The pycnocline presumably decouples the upper, wind-driven part of the water column from motion at greater depths, though this has not been documented with data from a properly designed study.

Summer heating, through characteristically clear to partly cloudy skies, takes over as the dominant physical process in July and August resulting in the formation and intensification of the seasonal thermocline. This form of stratification may also decouple the motion through the water column; however, more data are required to verify this possibility. At this time of year, the veering of surface winds into the southeasterly quadrant halts the south-southwesterly transport and produces instead a slowly alternating longshore current with a much slower speed.

There appears in some of the hydrographic data, evidence for an offshore transport in near-surface layers. This probably occurs as a small, cross-shelf deflection of a predominantly longshore current. Such a secondary circulation may explain the axis of the low-salinity plume over the middle shelf rather than against the coast (Figure 2.24) and the displacement of surface waters and a corresponding onshore encroachment of water at near-bottom levels (Smith, in Groover, 1977).

Lastly, the arrival of the first cold fronts of the season puts an end to (or at least largely skews) the alternating longshore motion and establishes the net transport to the south-southwest. At the same time, the seasonal thermocline is lost through surface cooling and motions in the unstratified water column become more vertically coherent.

Comments on the year-to-year repetition of physical processes in shelf waters must necessarily be restricted to hydrographic events. Appropriate current data are not available from previous years to provide a comparison. Several broad-scale features in the hydrographic patterns do appear to have been repeated in the 1977 data. Most notable are the recurrence of the low salinity plume of Mississippi River water in late spring and the spring cross-shelf temperature gradients characteristic of the late winter months. It is somewhat surprising that the small cyclonic loop in the T-S polygon appeared again in the 1977 data, but this may in fact be a quasi-permanent feature of the annual hydrographic cycle for Texas shelf waters, due to the interaction of the local precipitation and heating cycles.

The rapid warming during the late spring and early summer months and the most intense cooling occurring in the mid-Winter months suggest that more closely spaced sampling might be in order at these times of year to properly monitor the transitional periods. In this regard, it is unfortunate that the contract is based upon the calendar year as it expires at the start of one of the more interesting and important phases of the annual hydrographic cycle.

CONCLUSIONS

Hydrographic data and direct current measurements from the 1977 monitoring program revealed a predominantly annual progression of physical oceanographic variables. Net transport was to the south-southwest in the winter months; there was little net movement over time scales greater than a few weeks during the summer. Temperature and salinity measurements were used to trace out a clockwise loop. Annual temperature variations dominated the hydrographic climate of the South Texas OCS waters, but salinity variations were significant in late spring and early summer. The most important physical processes influencing Texas shelf waters were frontal passages in the fall and winter months, the spring runoff, and intense summer heating.

LITERATURE CITED

Armstrong, R. 1976. Historical physical oceanography; seasonal cycle of temperature, salinity and circulation. *In*: Environmental Assessment of the South Texas Outer Continental Shelf, 1975. NOAA Final Report (Draft), Vol. III, Oceanography, 27pp.

Bennett, A. 1976. Conversion of *in situ* measurements of conductivity to salinity. Deep-Sea Res. 23:157-165.

Etter, P., and J. Cochrane. 1975. Water temperature on the Texas-Louisiana shelf. Texas A&M Univ., TAMU-SG-75-604, 24pp.

Rivas, L. 1968. Fisherman's atlas of monthly sea surface temperatures for the Gulf of Mexico. U.S. Fish & Wildl. Serv., Bur. of Comm. Fisheries Circular 300, 33pp.

Smith, N. P. 1977. Hydrography Project. *In*: Environmental studies, South Texas outer continental shelf, biology and chemistry. R. D. Groover (ed.) pp 3-1 - 3-82, University of Texas. Final report to the Bureau of Land Management, Washington, D. C. Contract AA550-CT6-17

CHAPTER THREE

LOW-MOLECULAR-WEIGHT HYDROCARBON AND HYDROGRAPHIC PROJECT

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ABSTRACT

This report contains a comprehensive tabulation and analysis of all low-molecular-weight hydrocarbon (LMWH), dissolved oxygen, and nutrient data taken as part of the BLM South Texas Outer Continental Shelf (STOCS) program in 1977. The above mentioned parameters were measured at Stations 1-3, on the four transects during the three seasonal sampling periods (winter, spring and fall). In addition to these samplings, Stations 1-3, Trasect II were sampled during the six monthly sampling periods. At least three depths for LMWH analyses, dissolved oxygen and nutrients were sampled at each station. However, at most stations several additional samples were taken at intermediate depths to adequately define water column characteristics. Over 50 cores were also taken for LMWH analyses.

Water column LMWH were analyzed by a modification of the Swinnerton and Linnenbom (1967) method. Dissolved oxygen and nutrients were determined following the methods outlined by Strickland and Parsons (1972). Sediment LMWH were analyzed following the procedure of Bernard *et al.* (1978).

Methane showed considerable variation in the STOCS area waters. Near-bottom seepage was detected in bottom waters at some stations (*e.g.*, Station 3/IV). This seepage was also observed in sediment LMWH along the outer stations of Transect IV. The seepage along Transect IV may have been of petrogenic origin. A mid-water column methane maximum developed following stratification of the water column in summer and fall. Ethene and propene showed the same general trend as productivity; low surface values were observed in the winter with higher values in the spring, summer and fall. Ethene generally showed a maximum at some shallow depths (20 to 40 m) in the water column. The olefins generally dominated over their saturated analogs in the STOCS area. The lower Texas shelf is relatively "clean", as most of the light hydrocarbons on this shelf are presently derived from natural sources.

Oxygen values are controlled throughout most of the year in the upper 50 to 70 m of the STOCS area by physical processes. Nutrient concentrations were typically low, being representative of open Gulf surface water. Nitrate was limiting to productivity and disappeared during the summer and early fall. Phosphate and silicate were affected by the spring increase in productivity but were generally regenerated by fall. Increased continental runoff during the spring months was reflected by high silicate values at nearshore stations.

INTRODUCTION

Purpose

The purpose of the low-molecular-weight hydrocarbon analysis program was to establish levels for the C_1-C_4 hydrocarbon components in STOCS waters and sediments. These components are very sensitive indicators of spilled petroleum hydrocarbons and natural seeps. Once light-hydrocarbon levels have been established, a light-hydrocarbon monitoring program could indicate spilled oil and be a potential tracer of the more highly toxic components of petroleum.

Methane in unpolluted areas of the shelf is also a good indicator of suspended (organic) matter levels in the water column. Subsurface suspended matter profiles correlate well with methane profiles. An understanding of suspended matter variations is important since trace metals and petroleum hydrocarbons that are released during drilling can be adsorbed onto the surfaces of particulate material where they may be assimilated by filter and detrital feeders. Methane and other light hydrocarbon profiles in sediments and near-bottom waters may also indicate areas of gas seepage from the sediment-sea interface. These seeps may indicate the presence of subsurface oil or gas reservoirs. Characterization of hydrocarbon gas sources (biogenic versus petroleum-related) is made possible by determination of methane, ethane, and propane in near-surface sediments. High concentrations of interstitial gas may also affect sediment stability since gas saturation in sediments has been shown to destabilize sediments which can lead to failure of bottom-mounted structures.

The olefin levels (*e.g.*, ethene and propene) in the water column correlate with biological productivity parameters. Thus, the concentrations of these components in the water column are related to biological activity in the area.

The nutrient and dissolved oxygen concentrations are determined to support primary productivity measurements. Nutrient and dissolved oxygen concentrations are affected by the hydrology of a region as well as biological uptake and release. In turn, the concentrations of nutrients and dissolved oxygen in the water column greatly affect the species diversity and standing crop of phyto- and zooplankton. The benthic fauna are also affected to a lesser extent by the concentrations of these components in the water column.

Literature Survey

Water Column Low-Molecular-Weight Hydrocarbons

Dissolved gaseous hydrocarbons (methane through the butanes) in marine waters are derived from natural processes and/or man-related activities. Although microbially-produced methane has been known to exist in high concentrations (> 0.1 ml CH_4/ℓ) in anaerobic waters such as isolated fjords and basins (Atkinson and Richards, 1976; and Lamontagne *et al.*, 1973), the processes that control the concentrations of light hydrocarbons in the aerobic portion of the ocean have only recently begun to be understood. Prior to determinations by Swinnerton and Linnenbom (1967) of C_1-C_4 hydrocarbons in two open ocean profiles, there had been no published data for these components in the open ocean. The reported analyses of gaseous hydrocarbons in the open ocean have been mainly restricted to the authors' work over the past seven years in surveys of surface waters from over 5000 miles of cruise tracks and analyses of several thousand discrete samples (Brooks and Sackett, 1973; 1977; Brooks *et al.*, 1973, 1977; Sackett and Brooks, 1974, 1975; Brooks, 1975, 1976) and that of Naval Research Laboratory (Swinnerton and Linnenbom, 1967, Swinnerton *et al.*, 1969; Lamontagne *et al.*, 1971, 1973, 1974; and Swinnerton and Lamontagne, 1974). MacDonald (1976), and Scranton and Brewer (In Press) have recently reported light hydrocarbon concentrations in the Beaufort Sea and Eastern Tropical North Atlantic, respectively.

In the Gulf of Mexico, the author's laboratory has identified ports and estuaries with their associated commercial and petrochemical activities, offshore petroleum operations, and shipping activity, as the major man-derived sources of LMWH. Of these, the underwater venting of waste gases and brine discharges, both associated with offshore production platforms, are the major sources of non-methane light hydrocarbons to upper Gulf coastal waters. These sources are apparently responsible for the two orders of magnitude increase in Louisiana shelf waters over open ocean levels of the light hydrocarbons with average concentrations of 3100, 31 and 22 nl/ℓ of methane, ethane, and propane respectively (Brooks *et al.*, 1977).

Natural sources of light hydrocarbons to coastal waters include seepage of gas out of sediments and *in situ* production in the water column. The gas seeping from sediments can originate from either bacterial catalysis, involving the reduction of CO_2 or fermentation of organic compounds in anoxic environments, yielding principally methane or abiotic cracking, either thermal or catalytic, yielding a large spectrum of aliphatic and aromatic hydrocarbons. Processes controlling the *in situ* production in the water column of methane and olefins are not well defined in the literature.

Sediment Low-Molecular-Weight Hydrocarbons

These gases were analyzed in order to evaluate the relative contributions of microbially-produced and thermocatalytic gases in near-surface marine sediments. The STOCS was cored extensively in an attempt to locate areas of seepage of oil-related gas through faulting or bedding planes. Except for one area, no anomalously high concentrations of thermocatalytic natural gas were discovered on the STOCS. Therefore, the literature review on sediment gases will focus on the production and concentrations of microbial gas in marine sediments.

Bacteria obtain energy for growth and cell maintenance from a series of dehydrogenation, or coupled oxidation-reduction reactions. The uniqueness of each microorganism is based upon the type of molecule that can be used as the oxidizing agent or terminal electron acceptor. Two general types of metabolic processes are present in the decomposition of organic matter. One process uses inorganic compounds such as O_2, NO_3^-, $SO_4^=$, and HCO_3^- as electron acceptors (respiratory processes). The second process involves organic intermediates produced in the decomposition itself (fermentative processes). Although fermentation yields relatively little energy compared to aerobic and anaerobic respiration, they are not competitive and can occur simultaneously. In fact, fermentation processes can be preparatory steps for some respiration processes as they break down large organic molecules to small organic acids and alcohols.

The most efficient oxidizing agent is dissolved oxygen. During aerobic assimilation, oxygen is consumed by microorganisms until it is depleted. The bacteria then use other compounds capable or releasing energy when reactively coupled as oxidizing agents with organic matter. Table 3.1 (from Claypool and Kaplan, 1974) demonstrates the relative energy yields when glucose (representing organic matter) is oxidized by various agents. Actual free energy changes under natural conditions may vary but the advantage to organisms capable of utilizing the higher energy yield process is illustrated. When two or more physiologically different organisms compete for the same organic substrate, those capable of generating

TABLE 3.1

ENERGY-YIELDING METABOLIC PROCESSES AS COUPLED OXIDATION-REDUCTION REACTIONS (after Claypool and Kaplan, 1974).

	$\Delta G^{o}{}'$ (kcal per mole of glucose equivalent oxidized)
a. Aerobic respiration	
$CH_2O + H_2O \rightarrow CO_2 + 2H_2$	
$2H_2 + O_2 \rightarrow 2H_2O$	
$CH_2O + O_2 \rightarrow CO_2 + H_2O$	-686
b. Nitrate reduction	
$5CH_2O + 5H_2O \rightarrow 5CO_2 + 10H_2$	
$10H_2 + 4NO_3^- + 4H^+ \rightarrow 2N_2 + 12H_2O$	
$5CH_2O + 4NO_3^- + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O$	-579
c. Sulfate reduction	
$2CH_2O + 2H_2O \rightarrow 2CO_2 + 4H_2$	
$4H_2 + SO_4^= \rightarrow S^= + 4H_2O$	
$2CH_2O + SO_4^= \rightarrow S^= + 2CO_2 + 2H_2O$	-220
d. Carbonate reduction	
$2CH_2O + 2H_2O \rightarrow 2CO_2 + 4H_2$	
$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	
$CO_2 + H_2O \rightarrow HCO_3^- + H^+$	
$2CH_2O \rightarrow CH_4 + CO_2$	- 99
e. Nitrogen fixation	
$3CH_2O + 3H_2O \rightarrow 3CO_2 + 6H_2$	
$6H_2 + 2N_2 \rightarrow 4NH_3$	
$3CH_2O + 2N_2 + 3H_2O \rightarrow 4NH_3 + 3CO_2$	- 57
f. Fermentation: heterolactic	
glucose → acetaldehyde + CO_2 + lactate + H_2	
H_2 + acetaldehyde → ethanol	
glucose → lactate + ethanol + CO_2	- 49
g. Fermentation: Stickland reaction	
alanine + $2H_2O$ → NH_3 + acetate + CO_2 + $2H_2$	
$2H_2$ + 2 glycine → $2NH_3$ + 2 acetate	
alanine + 2 glycine + $2H_2O$ → 3 acetate + CO_2 + $3NH_3$	-17

the greatest metabolic energy will dominate.

The result of the oxidation efficiency succession of sediment ecosystems has been illustrated by Claypool and Kaplan (1974) by a schematic cross section of an organic-rich marine sedimentary column (Figure 3.1). The ecological factors form three distinct biochemical environments, each characterized by the dominant form of respiratory metabolism. The three zones distinguished by the authors were: the aerobic zone; the anaerobic sulfate-reducing zone; and the anaerobic carbonate-reducing zone.

Aerobic Zone

The aerobic zone is characterized by the dominance of microorganisms which use dissolved interstitial oxygen for respiration (aerobes). The processes which control interstitial oxygen concentrations are consumption by these bacteria, sediment deposition rate, and diffusion from overlying water. Oxygen concentrations necessarily approach zero at some sediment depth when diffusive processes can no longer replace the oxygen consumed by the aerobes. The depth of the aerobic-anaerobic boundary varies with sediment accumulation rates, organic content, and temperature. Sediments quickly become anoxic in nearshore and delta environments, and may contain no oxygen even at the surface. In continental shelf and slope regions, the oxidation of remobilized manganese demonstrated by bands of precipitated manganese dioxide illustrates the penetration of oxygen to depths of 20-40 cm. In the deep-sea sediments, oxygen may exist at several meters depth, as indicated by high Eh values in several Deep Sea Drilling Project cores (Presley *et al.*, 1970). These data are suspect, however, as recent measurements using O_2 microprobes have shown oxygen to essentially disappear at less than a meter depth even in abyssal sediments (C. Bowser, personal communication).

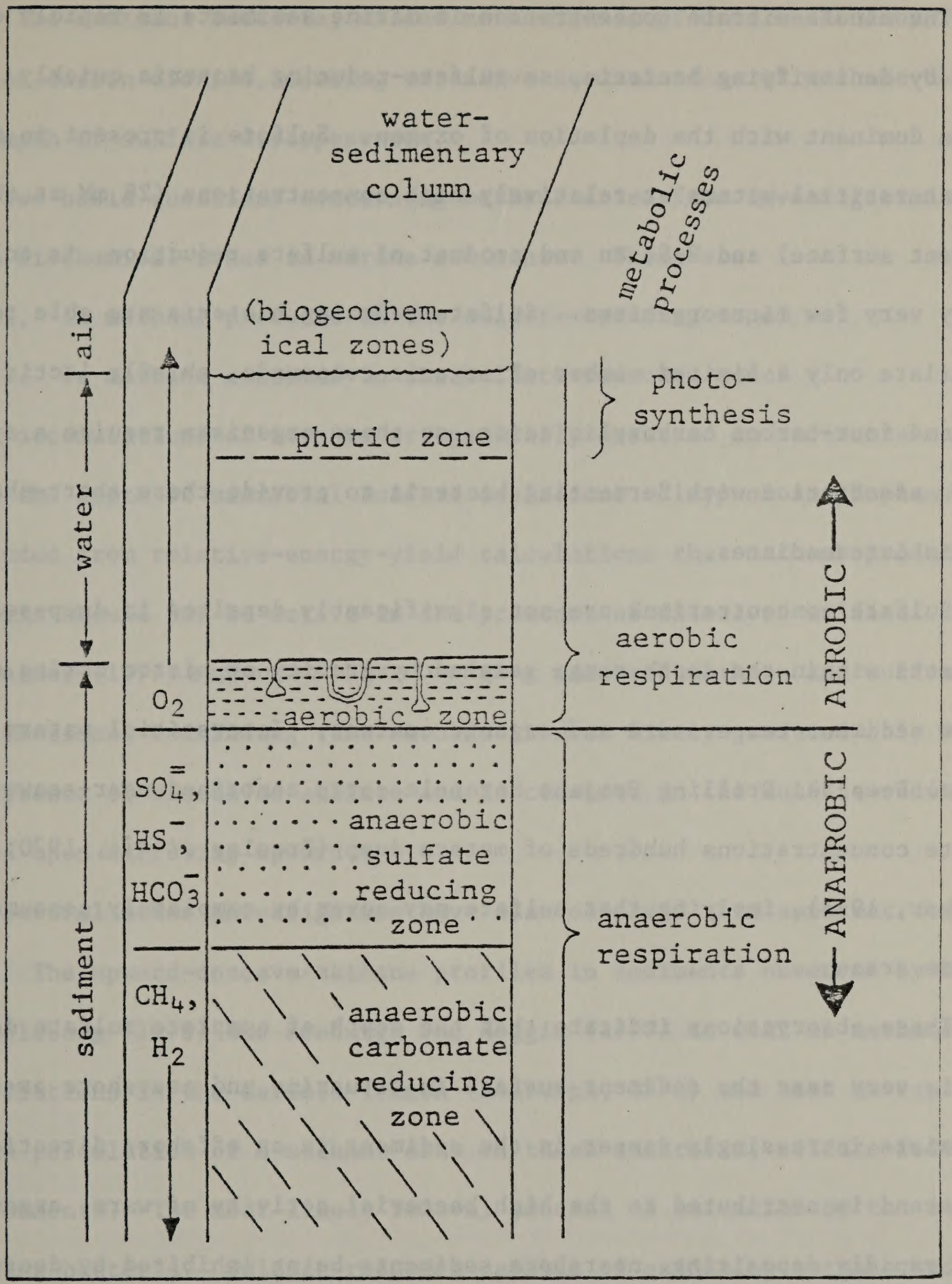

Figure 3.1 An Idealized Cross Section of an Open Ocean Marine Organic-Rich (Reducing) Sedimentary Environment (after Claypool and Kaplan, 1974).

Anaerobic Sulfate-Reducing Zone

The minute nitrate concentration in marine sediments is rapidly consumed by denitrifying bacteria, so sulfate-reducing bacteria quickly become dominant with the depletion of oxygen. Sulfate is present in marine interstitial waters at relatively high concentrations (28 mM at the sediment surface) and H_2S, an end product of sulfate reduction, is tolerated by very few microorganisms. Sulfate-reducing bacteria are able to assimilate only a limited number of organic compounds, chiefly lactic acid and four-carbon carboxylic acids, so these organisms require a symbiotic association with fermenting bacteria to provide these short-chain organic intermediates.

Sulfate concentrations are not significantly depelted in deep-sea sediments within the depth range sampled by gravity or piston coring due to low sediment temperature and organic content. Interstitial waters of several Deep Sea Drilling Project borehole cores contained near-seawater sulfate concentrations hundreds of meters deep (Presley *et al.*, 1970; Broecker, 1973), implying that sulfate may never by completely consumed in some areas.

These observations indicate that the depth of complete sulfate depletion is very near the sediment surface in estuarine and nearshore areas and exists increasingly deeper in the sediment in an offshore direction. This trend is attributed to the high bacterial activity of warm, organic-rich, rapidly depositing, nearshore sediments being inhibited by decreasing temperatures and organic input with increasing water depth.

Anaerobic Carbonate-Reducing Zone

This environment could more properly be termed the CO_2-reducing zone. When interstitial sulfate is depleted, microorganisms use the next most efficient electron acceptor available in marine sediments for respiration, CO_2. The dominant bacteria, called methanogens, produce methane by reduction of carbon dioxide, causing extensive accumulations of methane below the depth of sulfate disappearance.

Two basic questions concerning methane arise from investigations of these biochemical zones in marine sediments. These are:

1. Is methane produced in the sulfate-reducing zone?
2. Is methane consumed in the sulfate-reducing zone?

Large accumulations of methane are generally observed in sediments only below the depth of microbial sulfate depletion. Claypool and Kaplan (1974) concluded from relative-energy-yield calculations that methane-producing bacteria should not be active in the presence of dissolved sulfate. Martens and Berner (1977) supported this view using measured methane and sulfate distributions, concluding that methane is most likely produced only in the absence of dissolved sulfate and is consumed in the sulfate-reducing region upon diffusing upward.

Several other investigators have also observed this apparent consumption. The upward-concave methane profiles in sediments observed by Barnes and Goldberg (1976) and Reeburgh and Heggie (1977) as well as methane distributions in the Cariaco Trench (Reeburgh, 1976) can best be explained by the postulation of a methane sink in these anaerobic, sulfate-reducing environments. The most likely removal process is oxidation by the sulfate-reducing bacteria. Sulfate-reducers capable of oxidizing methane and other hydrocarbons using lactate as the principle carbon source have been cultured by Davis and Yarbrough (1966). In contrast, Sorokin (1957) could not detect methane consumption by sulfate-reducers when methane was the sole carbon source. This is not surprising, however, since there are no known anaerobic microorganisms capable of using methane as the sole carbon source (Quayle, 1972). Martens and Berner (1977) showed that methane could not be the chief carbon substrate for the sulfate reducers in anoxic marine sediments because too much sulfate is reduced per mole of methane consumed. Apparently, sulfate reducers prefer other organic compounds, but methane may be used as a secondary carbon source.

On the question of methane production in the sulfate-reducing zone, Barnes and Goldberg (1976) have suggested that methane generation and sulfate reduction are not mutually exclusive processes. Rather, low concentrations of methane in sulfate-reducing interstitial water represent a balance between production by methanogenic bacteria and consumption by sulfate reducers. This hypothesis implies that methane is produced in the sulfate-reducing zone at a rate comparable to that below the depth of sulfate depletion. Nearly all of the methane produced in the sulfate-reducing zone must then be oxidized to CO_2 to account for the observed low concentration. However, preliminary studies in our laboratory indicate that there is sufficient isotopic change in ΣCO_2 in this region for methane to be produced and consumed at such a high rate. Instead, methane may be produced to a limited extent in the sulfate-reducing zone, but at a much lower rate. As an example, Martens and Berner (1974) suggested that methane could be produced in the presence of interstitial sulfate within organic-rich, sulfate-free microenvironments such as the interior portions of decaying organisms.

Cappenberg has demonstrated an ecological relationship and an overlap in distributions of the sulfate reducers and methanogens from field observations in lake muds (Cappenburg, 1974a), inhibition experiments (Cappenburg, 1974b), ^{14}C-labeled substrate experiments (Cappenburg and Prins, 1974), and mixed continuous culture studies (Cappenburg, 1975).

Recently, Oremland and Taylor (1978) have found that the sulfate reducers and methanogens compete for available hydrogen so that when interstitial sulfate is reduced to a concentration low enough that it, rather than hydrogen, limits growth of sulfate reducers, methanogens can then use the available hydrogen for their growth. In other words, sulfate reducers dominate when sulfate is abundant and they effectively consume all available hydrogen produced in the sediments. When sulfate is no longer present in sufficient concentration to support cell growth of sulfate reducers, hydrogen becomes available to the methane producers.

Bacterial production of non-methane light hydrocarbons should also be mentioned. Whereas production of olefins (ethene and propene) by living marine organisms is commonly observed, attempts to detect microbially-reduced alkanes other than methane have generally failed. Davis and Squires (1954) reported the first detection of trace amounts of ethane produced from ethanol fermentation, but virtually no conclusive work since that early date has been accomplished. In summary, Coleman (1976) states, "It is not generally agreed by microbiologists that bacteria do not produce significant quantities of ethane (R.E. Kallio, personal communication)." The data presented in this report are in conflict with these beliefs.

Previous Work

Hydrographic

During the three years of the STOCS environmental assessment program, salinity, temperature, dissolved oxygen (DO), and nutrient data have provided ancillary information that has aided in the interpretation of other important biological and chemical parameters. Nutrient and dissolved oxygen concentrations are affected by hydrology and biological uptake and release. In turn, the concentrations of these components in the water column greatly affect the species diversity and standing crop of phyto- and zooplankton. The benthic fauna are also affected to a lesser extent by the concentrations of these components in the water column.

Nutrients (phosphate, nitrate, and silicate) and dissolved oxygen were taken at three depths in the water column (surface, half the depth of the photic zone, and near bottom) at Stations 1-3 along each of the four transects during seasonal samplings and along Transect II during monthly samplings. Samples were also taken near Southern Bank and Hospital Rock. Although nutrients and dissolved oxygen are generally quite variable spatially and seasonally, similar distribution patterns were observed over the first three years of the program. The work effort for 1977 presented here continued to provide these basic hydrographic parameters for the ongoing biological and chemical programs.

Water Column Low-Molecular-Weight Hydrocarbons

During the first contract year (1975) LMWH samples were taken at three depths in the water column from Stations 1-3, on all four transects, seasonally. The first year effort revealed areas of natural seepage in the STOCS region and distinct methane maxima at mid-depths during the spring and fall samplings. The second contract year (1976) LMWH were contracted to be analyzed at only two depths (surface and near-bottom) along the four transects seasonally and along Transect II monthly. However, many mid-depth analyses of methane were made so that the important subsurface maxima could be illustrated.

Sediment Low-Molecular-Weight Hydrocarbons

The measurements presented in this report represent the first reliable assessment of interstitial light hydrocarbon concentrations on the STOCS.

Virtually no measurements of interstitial hydrocarbon gas in open marine sediments exist in the present literature. This report serves to characterize concentrations of the LMWH in the STOCS area, as well as to point out one area of anomalous seepage of presumed thermocatalytic gas.

METHODS

Hydrographic

Samples for dissolved oxygen, nutrients, salinity and temperature for hydrographic measurements were taken by a series of Niskin or Nansen bottles. Dissolved oxygen samples were collected from Stations 1-3 of Transects I, II and III; Stations 1-6, Transect II; and from Hospital Rock and Southern Bank during the three seasonal sampling periods. In addition collections were made from Stations 1-6, Transect II during the six monthly sampling periods. Nutrient, salinity and temperature samples were collected from Stations 1-3, of all four transects, and from Hospital Rock and Southern Bank seasonally and from Stations 1-3, Transect II monthly.

Dissolved Oxygen

Water samples for dissolved oxygen were always drawn first from the sampling bottles so that the tendency toward equilibration of oxygen with the atmosphere was minimized. The oxygen flasks were filled by gravity flow from the sampling bottles through tygon tubing to the bottom of the flasks. The tubing was flushed of any bubbles before the flask was filled. The oxygen flasks were allowed to overflow approximately the volume of the flask and then stoppered. Dissolved oxygen samples were routinely taken and analyzed in duplicate. Samples were analyzed using the Winkler method, as outlined by Strickland and Parsons (1972) and modified by Carpenter. Concentrations were reported as milliliters (STP) of oxygen per liter seawater. Precision was somewhat dependent on the technician doing the analysis, but accuracy and precision were generally ± 0.02 ml/ℓ.

Nutrients

Three samples for nutrient determinations (phosphate, silicate, and nitrate) were also drawn from each of the sampling bottles. These samples were collected in 200-ml Whirl-Pak plastic bags and frozen until analysis. Samples were analyzed using an autoanalyzer following the methods outlined by Strickland and Parsons (1972). Specific methods for each of the nutrients were also given by Technicon Instruments Corporation of Ardsley, New York, Industrial Methods Bulletins 186-72W, (silicate), 158-71W, (nitrate), and 155-71W, (phosphate). Nutrient concentrations were reported as micromoles per liter seawater (μM).

Salinity

Samples for salinity were drawn from the sampling bottles into 500-ml citrate bottles that had been rinsed twice with sample water. Salinity was determined in duplicate on a Plessey 6210 inductive salinometer. Salinity was reported as parts per thousand (°/₀₀) of dissolved solids by weight. Accuracy and precision were typically ± 0.001 °/₀₀.

Temperature

Temperatures were read in duplicate from two reversing thermometers attached to each sampling bottle, and reported as degrees centrigrade. These thermometers underwent periodic calibration to ± 0.005°C by our Marine Operations section and most thermometers had a long calibration history. Temperature measurements were typically accurate to ± 0.01°C.

Dissolved Low-Molecular-Weight Hydrocarbons

LMWH samples were collected from Stations 1-3, on all transects, during the three seasonal sampling periods. Additionally, Stations 1-3, Transect II were sampled during the six monthly sampling periods. Although an attempt was made to sample the area synoptically, the physical sampling of the twelve stations took from six days (*e.g.* fall seasonal) to over six weeks (*e.g.* winter seasonal). Thus, rigorous interpretations of areal distributions should take sampling periods into account. The monthly samplings of Transect II required no more than three days.

Samples were taken by standard hydrographic casts using a series of Niskin or Nansen bottles. After retrieval, the seawater samples were transferred by gravity flow into 1-liter ground-glass stoppered bottles. The bottles were capped in such a way as to avoid entrapment of gas bubbles. The samples were poisoned with sodium azide to prevent bacterial alteration.

The 1977 contract called for LMWH samples to be collected at three depths (surface, one-half the depth of the photic zone, and near-bottom) at each station. The LMWH (methane, ethene, ethane, propene and propane) were determined by a modification of the Swinnerton and Linnenbom (1967) method. Seawater samples were purged directly from the sample bottles by a hydrocarbon-free helium stream. The purge helium was purified by a charcoal trap at liquid nitrogen temperatures. After collection the trap was isolated, heated by a water bath (90°C), and then injected into the gas chromatographic stream for analysis. The LMWH were separated on a 1.8-m 3.0-mm Outside Diameter (OD) Poropak Q column, analyzed with a Flame Ionization Detector (FID) and reported as nannoliters per liter seawater (nl/ℓ). Sensitivity of the method was 0.1 nl hydrocarbon /ℓ seawater and precision was generally better than ±5%.

In addition to surface and near-bottom samples, intermediate depths at most stations were sampled for LMWH analysis. These supplemental samples were taken in 125-ml narrow mouth bottles with screw top caps. The bottles were poisoned and stored upside-down until analysis. They were analyzed by McAullife's (1971) method of multiple phase equilibrium. McAullife's method involved equilibrating (by shaking) 25 ml of purified helium with 25 ml of sample water in a 50 ml syringe with a Luer-Lok stopcock. Since >96% of the light aliphatic hydrocarbons partitioned into the gas phase, analysis was performed by injecting part of the equilibrated gas phase into the chromatographic stream by means of a sample injection valve. These intermediate samples provided more detailed vertical distributions of methane and ethene, but since the sensitivity of the method was approximately ±5 nl/ℓ of seawater, few of the other C_2 and C_3 hydrocarbon levels were measured at intermediate depths.

Sediment Low-Molecular-Weight Hydrocarbons

Methods for all of the previously mentioned parameters have been outlined very briefly because detailed procedures for each have been published elsewhere. Procedures for determination of sediment low-molecular-weight hydrocarbons have only recently been developed. To date, three different approaches have been used: 1) Interstitial waters have been squeezed from sediment sections and the dissolved gases determined after being stripped from the pore water; 2) Gases have been stripped and analyzed from interstitial water collected in tubes at variably spaced depths on a gas harpoon, or *in situ* sampler, which has been driven into the sediment; 3) Gases have been stripped and analyzed from whole sediment sections which have been preserved to prevent gas escape. The procedure developed by the authors' laboratory is similar to the third method and presented in detail here.

Sediment samples were obtained using standard gravity coring techniques.

Upon retrieval the sediment contained in a plastic liner was removed from the core barrel and sectioned at specific depths. Five-centimeter sections were immediately extruded into 0.5-ℓ containers containing 125 ml of sodium azide-poisoned, hydrocarbon-free seawater. The containers were capped and the headspaces flushed with helium or nitrogen through septa in the lids. The light hydrocarbons dissolved in the interstitial water were equilibrated with the gas phase by agitation for five minutes with a high speed shaker. The shaker also dispersed the sodium azide throughout the sediment to inhibit microbial activity. The head space gases were then analyzed or the containers inverted to form liquid seals around the lids and stored in darkness at near-freezing temperatures until analysis.

The system used for analysis of the sediment LMWH is shown schematically in Figure 3.2. Trap A contained activated charcoal maintained at liquid nitrogen temperature for removal of hydrocarbon impurities in the purge helium stream. The system was flushed by opening all values and heating trap B to ~ 90°C with a boiling water bath. Trap B contained Porapak Q as a substrate to collect the hydrocarbons. Liquid nitrogen was placed around the trap and valve C closed before the container was coupled to the system by inserting 20-gauge needles into the septa (outflow line first). Helium entered the sample container through valve D, purged the headspace gases through an anhydrous magnesium perchlorate drying tube, and carried the light hydrocarbons into trap B where they were quantitatively collected.

The flush rate was adjusted by valve D to one liter per minute so that the hydrocarbons in the 0.2 ℓ headspace were quantitatively removed in two minutes. The trap was then isolated by closing valve F and G, and the container removed from the system. The trap was then

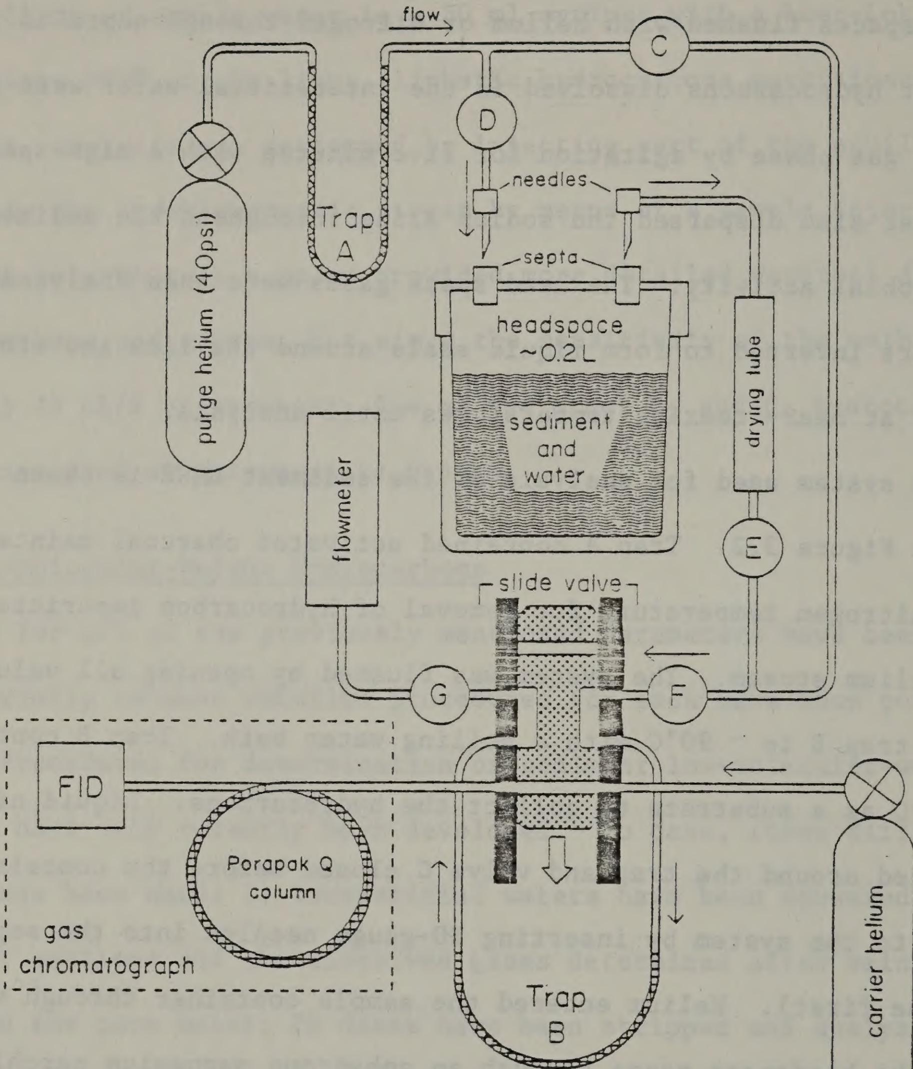

Figure 3.2 Schematic of the System Used for Analysis of Sediment Light Hydrocarbons.

heated, injected into the carrier stream by a pneumatic slide value, and the hydrocarbons separated on a 3 m, 1.5-mm inside diameter (I.D.) Porapak Q column thermostated at 60°C. A flame ionization detector was used in conjunction with an electronic integrator for the analysis of sediment LMWH concentrations. A typical chromatogram showing separation of the gases is shown in Figure 3.3.

Due to the solubility differences of the light hydrocarbons in seawater, the partition coefficients between the water-sediment mixture and headspace varied for each hydrocarbon. Partition coefficients for the individual hydrocarbons were determined by repeated equilibrations of samples after replacement of the headspace gas with helium. After purging, the sample container was re-agitated and the analysis procedure repeated. The individual partition coefficients were calculated by the equation:

$$K_i = 1 - (X_2/X_1)_i \qquad (1)$$

where K is the partition coefficient for a particular hydrocarbon, and $(X_2/X_1)_i$ is the ratio of the detector response generated by component i from the first (X_1) and second (X_2) equilibrations. These partition coefficients represented the fraction of total gas in a sample container that was present in the gas phase after an equilibration. Since at least 80% of every light hydrocarbon gas was removed from the sediment by each equilibration, simply summing the response from the first two equilibrations represented at least 96% recovery of each gas from the sediment samples. Since coefficients for a group of cores taken and analyzed under similar conditions were quite repetitive, it was faster and more accurate to establish standard partition coefficients of each gas in a group of core samples by performing second equilibrations only on selected samples. The total response of a gas in a sample (T_i) could then be calculated using the

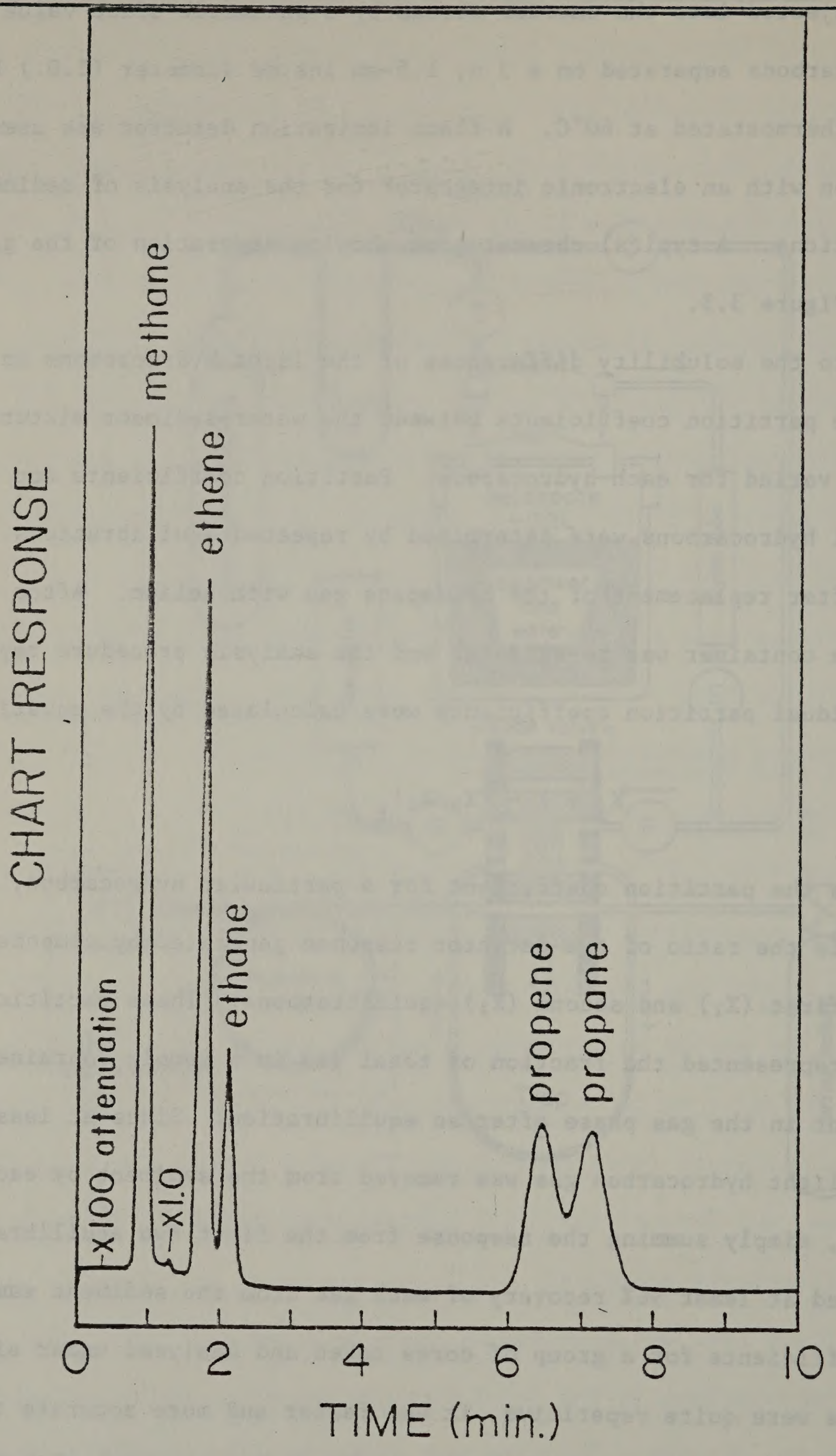

Figure 3.3 Chromatogram of Sediment Light Hydrocarbons.

equation:

$$T_i = (X_1/K)_i \quad (2)$$

After a volume of a standard gas mixture was trapped and injected into the gas chromatograph for calibration, concentrations of each gas could be calculated by the equation:

$$C_i = (C_{std}/X_{std})_i \times (V_{std}/V_{mud}) \times T_i \quad (3)$$

where gas concentration (C_i) is in microliters per liter wet sediment, C_{std} is the concentration of component i in a standard gas mixture in parts-per-million, X_{std} represents detector response generated by standard component i, V_{std} is the volume of standard gas in milliliters, and V_{mud} is the volume in milliliters of sediment placed in the sampling container.

As an experimental verification of the analytical procedures, a sediment sample was equilibrated and purged five times to remove all measurable LMWH. A 20cc sample of standard gas containing quantitites of LMWH in the range of those typically measured in continental shelf sediment samples was injected into the container. The container was then agitated at room temperature to equilibrate the hydrocarbons and headspace gases were measured as previously outlined. After purging, the container was again equilibrated and the new headspace gases measured. The data generated by the experiment are presented in Table 3.2. For each hydrocarbon gas, the table lists the number of nannoliters contained in 20 cc of the standard mixture, the integrator units generated from the first and second equilibrations of the container, and the resulting partition coefficients. Also tabulated were the integrator units measured from direct analysis of the hydrocarbons in 20cc of the standard gas (these represent total units which were injected into the container) and the total units in the sample. Percentage

TABLE 3.2

INTEGRATOR UNITS OBTAINED IN GAS PARTITIONING EXPERIMENTS

	Methane	Ethene	Ethane	Propene	Propane
Nanoliters of gas injected	380	16	17	15	18
Response, first equilibration (X_1)*	619.8	51.57	60.51	72.09	91.89
Response, second equilibration (X_2)	37.25	7.425	4.039	9.313	3.966
Calculated partition coefficient (K)	0.940	0.856	0.933	0.871	0.957
Calculated total units in sample (T)	659.4	60.2	64.9	82.8	96.0
Response, calibration standard (X_{std})	666.5	59.2	65.3	81.7	96.5
Percent deviation of T from X_{std}	-1.1	1.7	-0.6	1.3	-0.5

*Symbols are explained in text.

deviations of the calculated and measured values showed that there was less than a 2% error for every hydrocarbon by calculating concentrations from partition coefficients. Therefore, if consistent partition coefficients were established for a large group of samples, most of the samples analyzed needed to be equilibrated only once.

Average partition coefficients calculated from two large groups of sediment samples taken from the Texas continental shelf and slope are presented in Table 3.3. Both groups were stored in a refrigerator, but Group A was warmed to room temperature (20°C) before analysis, whereas Group B was heated to about 40°C in a hot water bath. The gas solubilities in 20°C distilled water calculated from the data of McAuliffe (1966) were also tabulated for comparison. Solubilities of most of these gases in seawater are not accurately known but should follow the same trend as in distilled water. Partition coefficients are a function of the solubilities of gases which, in turn, depend on temperature. The coefficients listed in Table 3.3 reflect the relative gas solubilities, decreasing with increasing solubility. Group B partition coefficients were noticeably higher than those of Group A, indicating the negative effect of higher temperature on gas solubilities. Higher partition coefficients decreased the chance of error in the calculation of total gas in the samples because relatively more gas was removed from the sample during the first equilibration, so warming the samples to 40°C was the preferred procedure.

It was also discovered after the majority of the samples for this work were analyzed that the glass containers containing the sediment-water mixture would not break if frozen. All subsequent samples were frozen before analysis for two reasons: 1) microbial activity should be completely inhibited, and 2) the crystallization and expansion of the water during freezing tends to break up the structure of the clay particles, thus helping expose

TABLE 3.3

PARTITION COEFFICIENTS OF TWO GROUPS OF SAMPLES

	Methane	Ethene	Ethane	Propene	Propane
Group A (20°C)	.944	.840	.897	.835	.950
Group B (40°C)	.955	.848	.919	.902	.960
*Solubility @ 20°C (ml/l)	34	105	45	107	32

*Calculated from data of McAuliffe (1966) any LMWH occluded on or trapped inside the clay lattice. Indeed, higher apparent partition coefficients for all the light hydrocarbons, especially ethane and propane, were observed from analysis of frozen samples.

For each section of sediment sampled for LMWH, an adjacent sample of sediment was collected, weighed, freeze-dried, and reweighed for the determination of weight percent interstitial water. From this percentage, porosity could be calculated and concentrations of sediment LMWH were reported per liter interstitial water rather than wet sediment.

For measuring methane concentrations in excess of saturation at one atmosphere pressure, such as are typically found in sulfate-free reducing sediments, the sampling method outlined here is inferior to the so-called *in situ* pore water samples (or gas harpoons) developed by other investigators, because of the possibility of outgassing during core retrieval. However, existing *in situ* samplers cannot collect enough pore water for precise determinations of light hydrocarbons other than methane. The sediment depth which can be reached and the sampling intervals are also limited with the *in situ* technique. Concentrations of hydrocarbon gas existing in the top few meters of the Texas continental shelf and slope sediments are far below saturation so that the escape of gas during handling by our sampling method was driven only by the processes of molecular diffusion from the core material. The time period that the core material was exposed to conditions causing loss of gas due to outward diffusion after extrusion was generally less than one minute. The depth within the core section to which significant gas loss occurred during this time was estimated using a diffusion coefficient of 2×10^{-6} cm^2/sec (Martens and Berner, 1977) to be about 0.1 mm. In effect then, in one minute diffusive processes skimmed the outer 0.1 mm from the surface of the exposed core section, introducing a maximum reduction of one percent in total gas content of a sample.

RESULTS AND DISCUSSION

Water Column Low-Molecular-Weight Hydrocarbons

Table 3.4 shows the number of observations, mean, minimum, and maximum values for each of the LMWH measured in the STOCS region in 1977. These values are tabulated in Appendix B, Tables 1 through 24.

Areal Distribution of Methane

Figure 3.4 shows near surface methane concentrations during the seasonal cruises in the STOCS area in 1977. The processes controlling the surface concentrations of methane on the STOCS appeared to be similar to those occurring in the open ocean waters of the Gulf of Mexico. In the open Gulf of Mexico, concentrations are controlled mainly by biological processes occurring in the water column and exchange across the air-sea interface. Biological and physical processes (*e.g.* salinity and temperature variations) are more pronounced in the coastal zone resulting in greater variability than observed in the open Gulf. The lower Texas shelf is generally free of the large man-derived additions that control surface methane and other LMWH concentrations on the upper Texas-Louisiana shelf. These man-related sources include offshore drilling and production, transportation losses, river runoff, and inputs associated with bays and harbors. The STOCS region is relatively clean with respect to man-induced LMWH since large scale drilling and production has not commenced on this part of the Texas shelf.

Surface concentrations of methane on the STOCS are influenced by air-sea exchange. If there were no man-derived or natural inputs of methane, surface concentrations would be at equilibrium with the atmosphere. The

TABLE 3.4

SELECTED PARAMETERS IN THE STOCS (1977)*

Variable	Number of Observations	Mean	Minimum	Maximum
Methane (nl/ℓ)	411	339	39	5,410
Ethene (nl/ℓ)	387	4.8	0.11	20.5
Ethane (nl/ℓ)	356	1.7	0.12	58.5
Propene (nl/ℓ)	255	1.0	0.25	2.8
Propane (nl/ℓ)	251	0.9	0.20	16.6
R (methane)	323	6.1	1.11	104
Depth (m)	528	37.9	0	130
Temperature (°C)	414	22.7	9.62	30.16
Salinity (°/₀₀)	349	35.47	27.946	36.679
Oxygen (ml/ℓ)	524	4.89	2.90	6.95
R (oxygen)	346	1.0	0.56	1.16
Phosphate (μM)	406	0.21	0	4.74
Nitrate (μM)	419	1.0	0	18.1
Silicate (μM)	419	2.7	0.10	13.9

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*Includes Topographic Features Study (BLM Contract AA550-CT7-15)

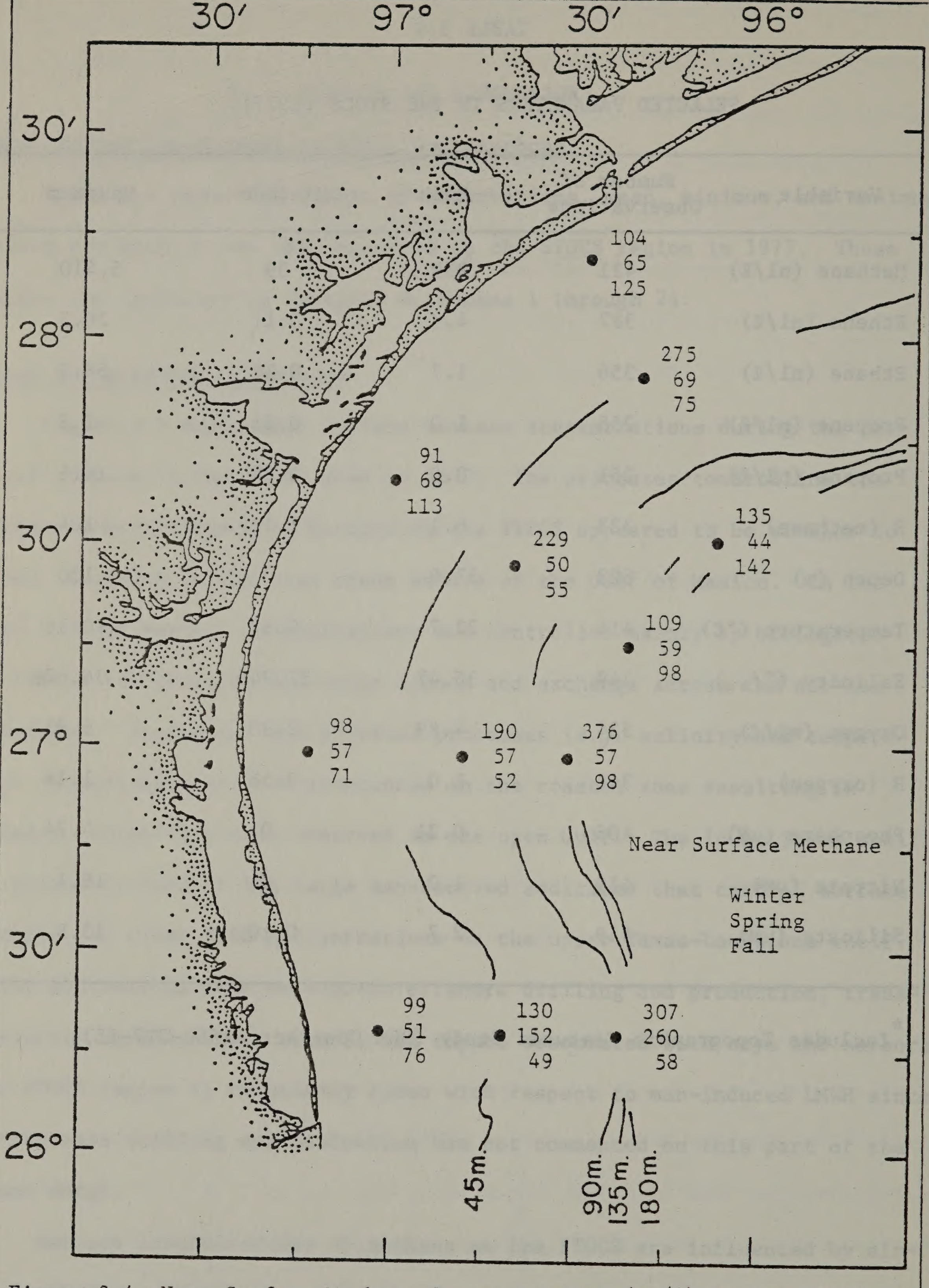

Figure 3.4 Near-Surface Methane Concentrations (nl/ℓ) in the STOCS During the Seasonal Cruises in 1977.

concentration of a dissolved non-reactive gas in seawater is determined according to Henry's Law by its solubility coefficient at the temperature and salinity during water mass formation and its partial pressure in the atmosphere. For the LMWH, only the partial pressure of methane (1.4 ± 0.1 ppmv for the atmosphere over the entire earth) is known with any degree of certainty. Using this value and the Bunsen solubility coefficients reported by Yamamoto *et al.* (1976), the equilibrium solubility of methane in the water column at the existing temperature and salinity has been computed for each observation. Also computed for each observation was an R value, where R is the ratio between measured and equilibrium methane concentrations, with R greater than 1.0 indicating supersaturation. The R values (Table 3.4) for methane ranged from 1.11 to 104 times equilibrium concentrations in the water column (mean = 6.1; 323 observations).

Figure 3.5 shows R values of near-surface methane concentrations during the seasonal cruises in the STOCS area. Although higher values were usually observed at nearshore stations, there were no seasonal or spatial patterns observed in these surface samples. There were 54 surface observations for methane in the STOCS area during 1977 which ranged from 1.19 to 9.26 times equilibrium concentrations and averaged 1.81. Thus the surface waters in the STOCS area showed a consistent surface supersaturation with respect to the partial pressure of methane in the atmosphere.

These observations agree with the reports of several other investigators (Lamontagne *et al.*, 1971; 1973; Brooks and Sackett, 1973, 1977; Swinnerton and Lamontagne, 1974; Brooks, 1975; and Scranton and Brewer, In Press) which show that the mixed layer of the ocean is supersaturated with respect to the partial pressure of methane in the atmosphere. The supersaturation appears to be a permanent feature of the world oceans, except in the regions of strong upwelling such as the Yucatan Shelf (Brooks *et al.*, 1973) and in

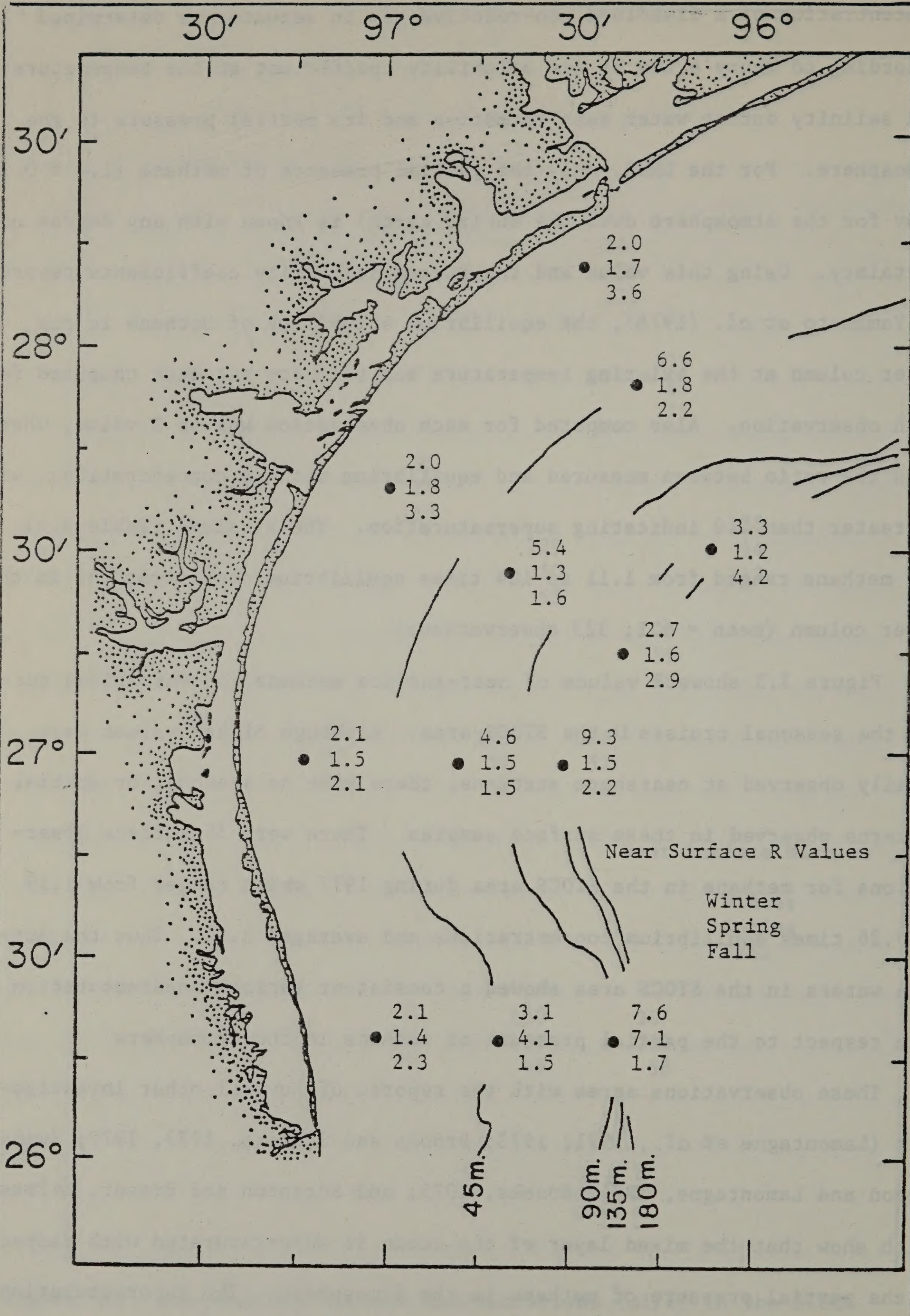

Figure 3.5 Near-Surface R (Degree of Saturation) Values for Methane in the STOCS During the Seasonal Cruise in 1977.

some ice-covered areas of the Antarctic (Lamontagne *et al.*, 1974). Sources of the excess methane in coastal water include offshore production operations, runoff, transportation activities, and diffusion from methane-rich sediments. The source of the excess methane in the open ocean has been attributed to *in situ* production in the water column (Lamontagne *et al.*, 1973; Brooks and Sackett, 1973; Seiler and Schmidt, 1974; and Scranton and Brewer, In Press). Since man-derived additions of LMWH on the STOCS do not, at present, appear to be affecting LMWH levels, the excess methane on the STOCS must come from *in situ* production, diffusion out of methane-rich sediments, or gas seepage.

Vertical Methane Distribution

The vertical distribution of methane in the STOCS region exhibited both seasonal and spatial variations. Table 3.5 shows the number of observations, the average mean water column concentration, the average surface concentration, and the minimum and maximum values for each of the three seasonal and six monthly samplings. Table 3.6 shows the surface and near-bottom methane concentrations at STOCS stations along Transect II. There did not appear to be any discernible seasonal pattern in surface methane concentrations along Transect II, although higher surface methane concentrations were observed during the December sampling at all stations. Station 1/II generally had higher surface methane levels than the stations farther offshore, no doubt due to the close proximity of the sediment-water interface and coastal contributions (*e.g.* runoff). Higher surface methane concentrations were associated with Station 1, Transects I and II than along Transects III and IV, probably due to more river and estuarine runoff into these nearshore areas.

The vertical distribution of methane showed large seasonal variations at intermediate depths (30 to 100 m) in the water column over the 1975 to

TABLE 3.5

SUMMARY OF NUMBER OF METHANE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (in nl/) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1977).

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	62	198	77	825	178.6
March	9	155	65	403	88.3
April	16	192	47	519	92.3
Spring	78	263	41	1280	82.4
July	22	524	48	4000	230.3
August	19	124	44	391	48.7
Fall	86	253	49	1800	82.7
November	19	146	49	354	81.0
December	17	163	75	341	103.7

TABLE 3.6

SURFACE AND NEAR-BOTTOM METHANE CONCENTRATIONS (nl/) AT STOCS STATIONS ALONG TRANSECT II.

SAMPLING MONTH	STATION 1/II	2/II	3/II
Surface			
Winter	91	229	109
March	90	110	65
April	121	108	59
Spring	68	50	59
July	578	65	48
August	44	50	52
Fall	113	55	98
November	75	119	49
December	97	75	139
Near-Bottom			
Winter	99	241	250
March	127	223	403
April	208	480	519
Spring	145	194	439
July	510	185	116
August	391	116	209
Fall	98	154	288
November	98	132	308
December	96	124	212

1977 sampling period in the STOCS. In 1975 the water column was fairly uniform with respect to methane during the winter sampling. A large maximum (up to 4000 nl/ℓ) was observed from the spring sampling at intermediate depths of several stations. This same maximum, although at much lower concentrations, was also observed from the fall sampling in 1975. In 1976, a similar but not identical pattern developed. No concentrations in the thousands of nl/ℓ methane were observed in 1976, as was the case in 1975.

In 1976, the winter sampling of all deep stations (Stations 3/I, 3/II, 3/III and 3/IV) showed a fairly uniform methane distribution in the water column with the exception of Station 3/II. The March sampling at Station 3/II showed that a broad methane maximum had developed in the 50 to 80 m depth interval. This maximum was absent during the April, spring and July 1976 samplings of this stations. The maximum was again present during the August sampling at the 70 to 100 m depth interval. Although no mid-depth samples were taken during the fall sampling, near-bottom concentrations at Station 2/II during the fall 1976 seemed to indicate the presence of the mid-depth maximum. It was also present during the November 1976 sampling, although insufficient sample depths were obtained to define its extent. In December, a uniform water column with respect to methane had again developed.

In 1977, large mid-depth methane concentrations were again observed in the STOCS (Figures 3.6 to 3.14). These maxima appeared to develop with formation of a thermocline in April (Figure 3.8). The maximum became pronounced at 60-70 m during spring, July and fall (Figures 3.9, 3.10 and 3.12) sampling periods. As in 1975, concentrations as high as 4000 nl/ℓ were observed within this maximum (Figure 3.10) during July. The maximum was deeper (80 to 100 m) and therefore only present at Station 3/II during the August and November samplings (Figures 3.1 and 3.13).

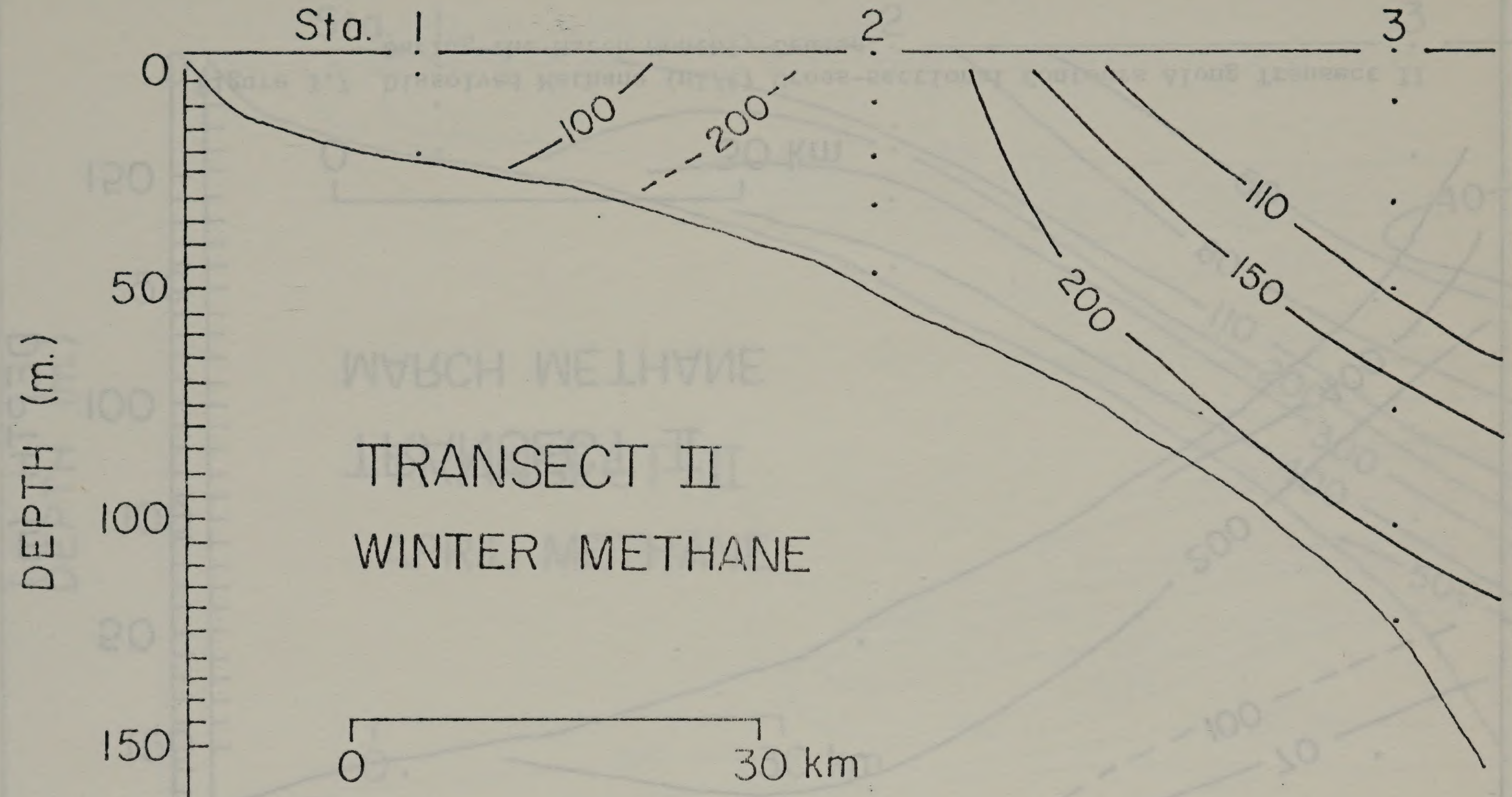

Figure 3.6 Dissolved Methane (nl/ℓ) Cross-sectional Contours Along Transect II During the March Monthly Cruise.

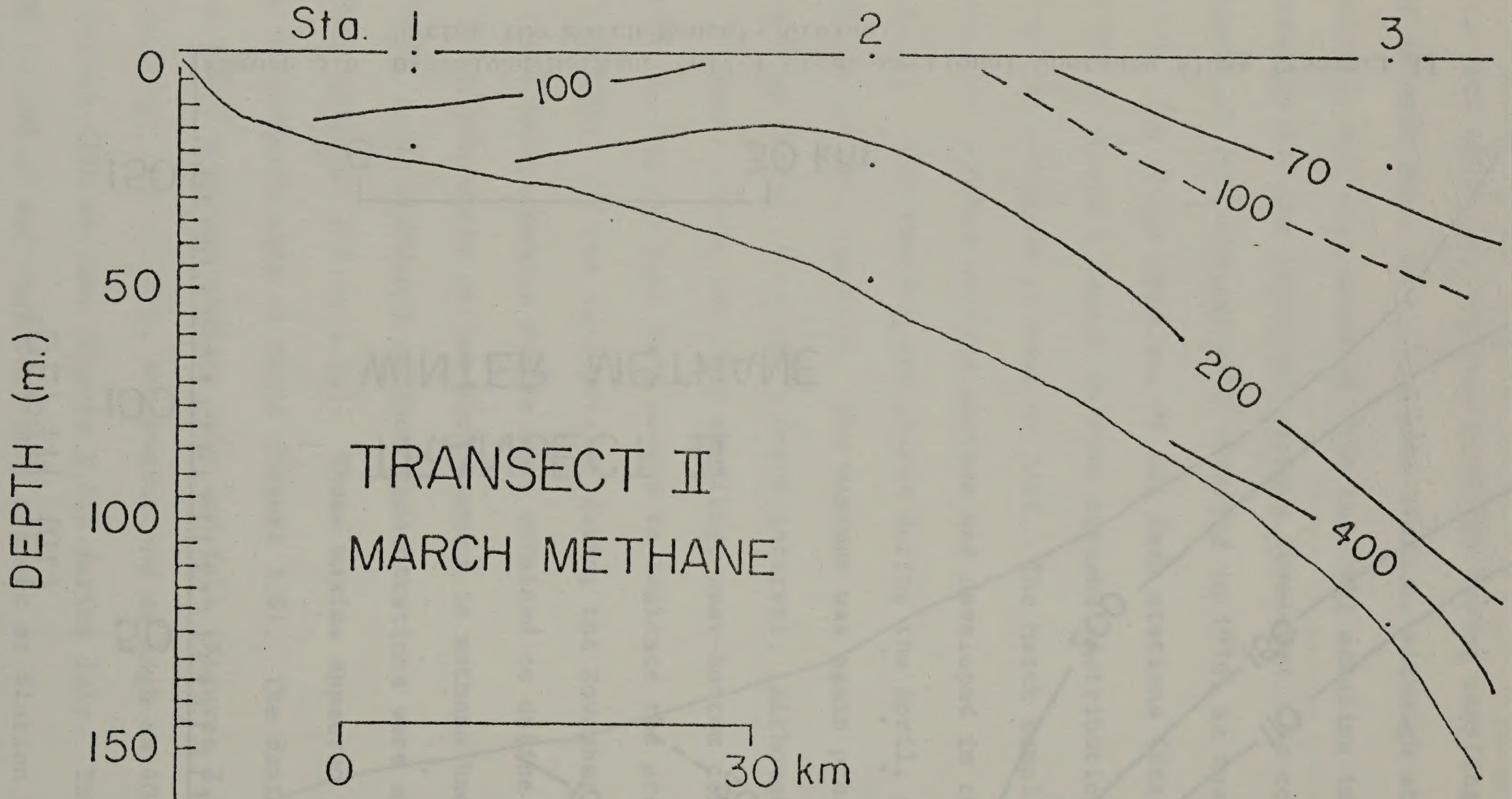

Figure 3.7 Dissolved Methane (nl/ℓ) Cross-sectional Contours Along Transect II During the March Monthly Cruise.

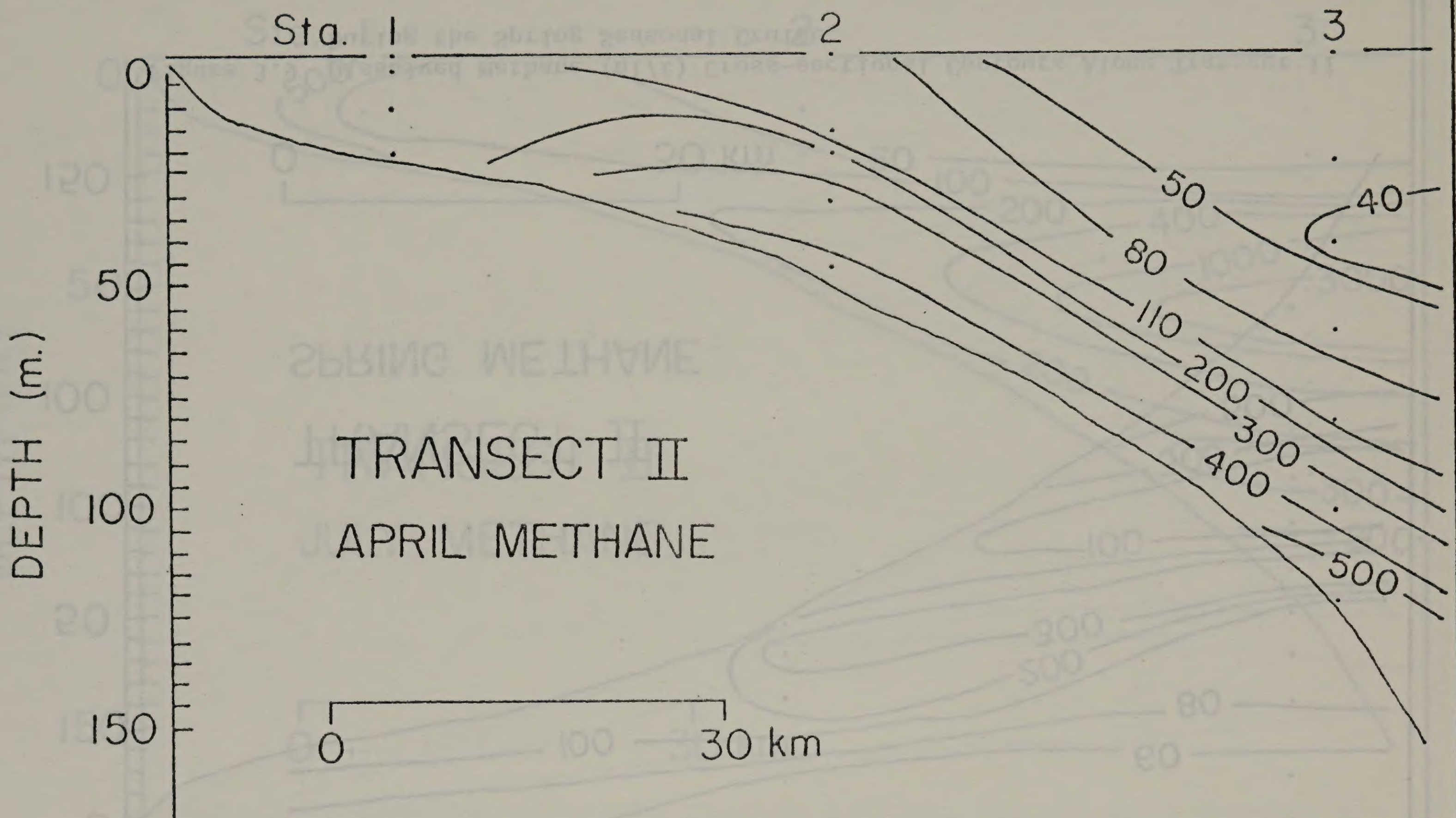

Figure 3.8 Dissolved Methane (nl/ℓ) Cross-sectional Contours Along Transect II During the April Monthly Cruise.

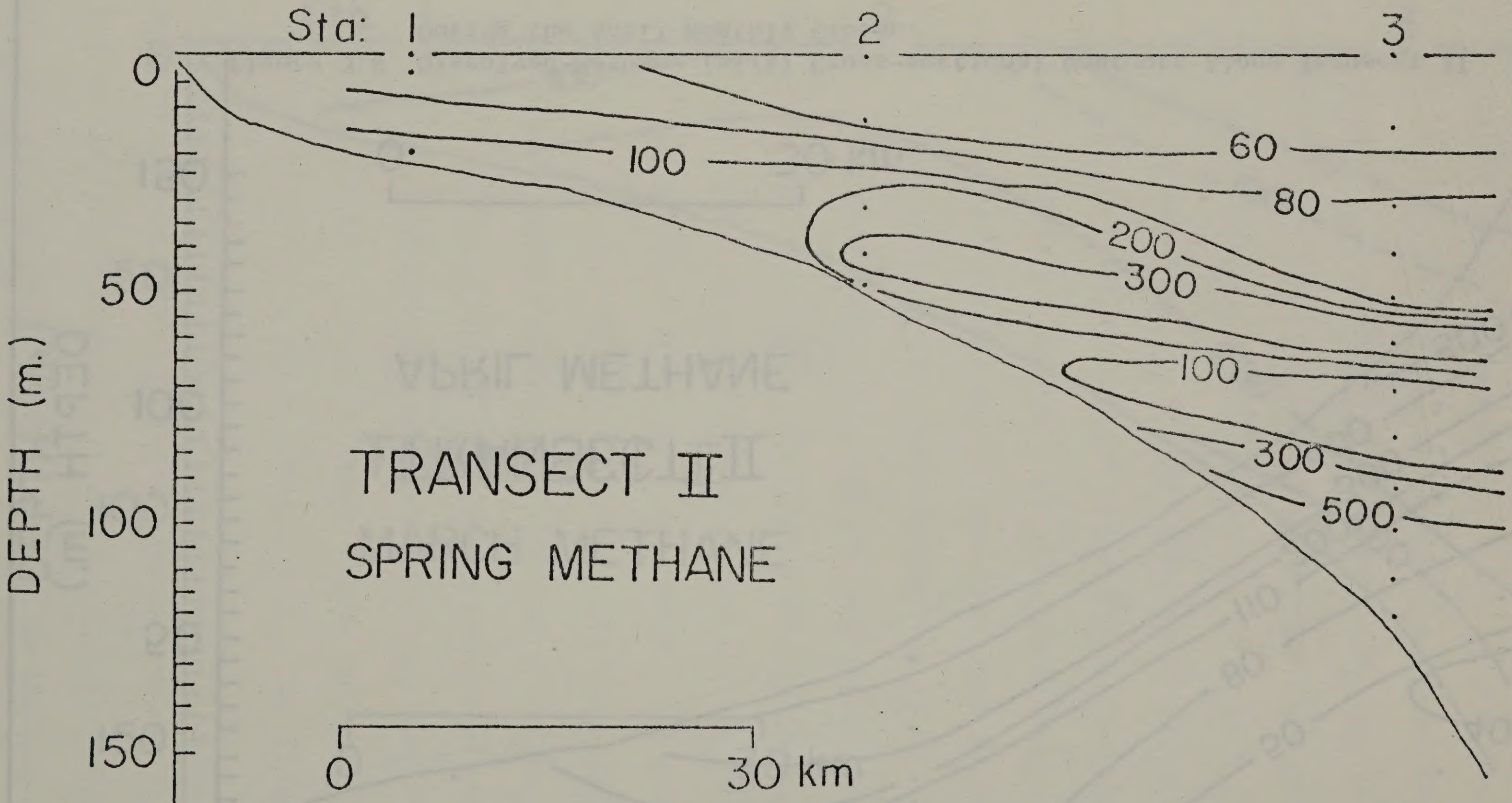

Figure 3.9 Dissolved Methane (nl/ℓ) Cross-sectional Contours Along Transect II During the Spring Seasonal Cruise.

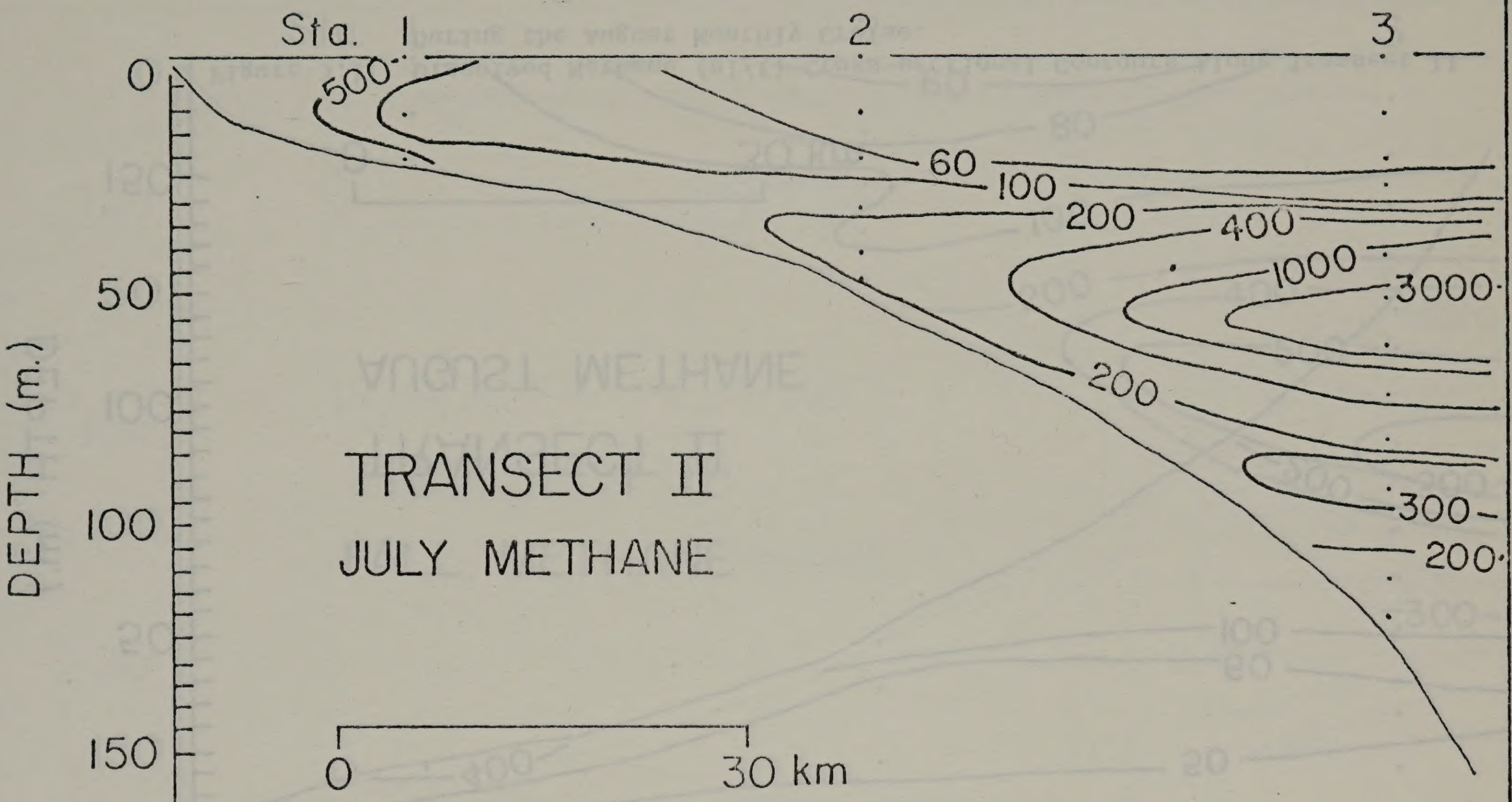

Figure 3.10 Dissolved Methane (nl/ℓ) Cross-sectional Contours Along Transect II During the July Monthly Cruise.

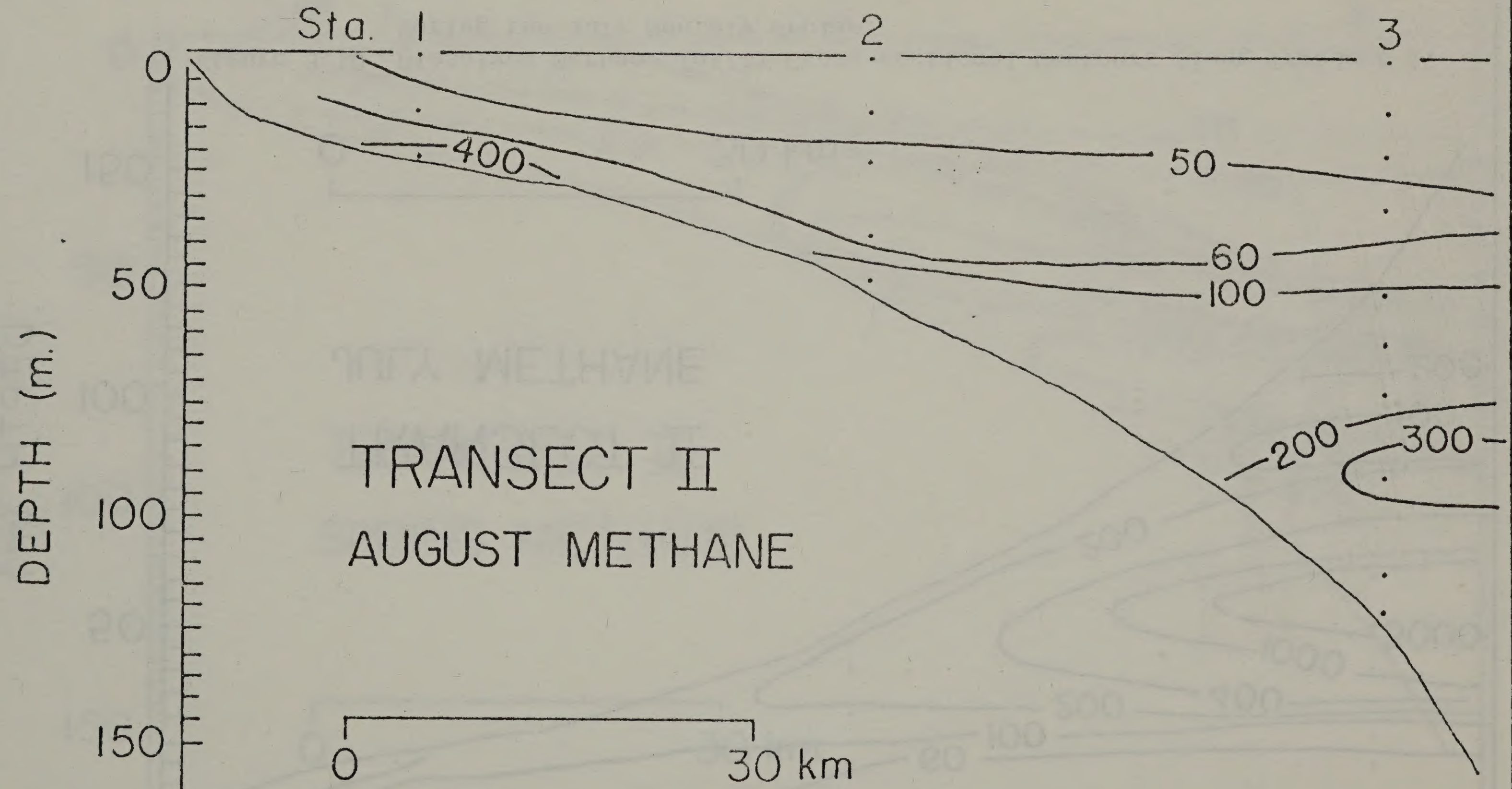

Figure 3.11 Dissolved Methane (nl/ℓ) Cross-sectional Contours Along Transect II During the August Monthly Cruise.

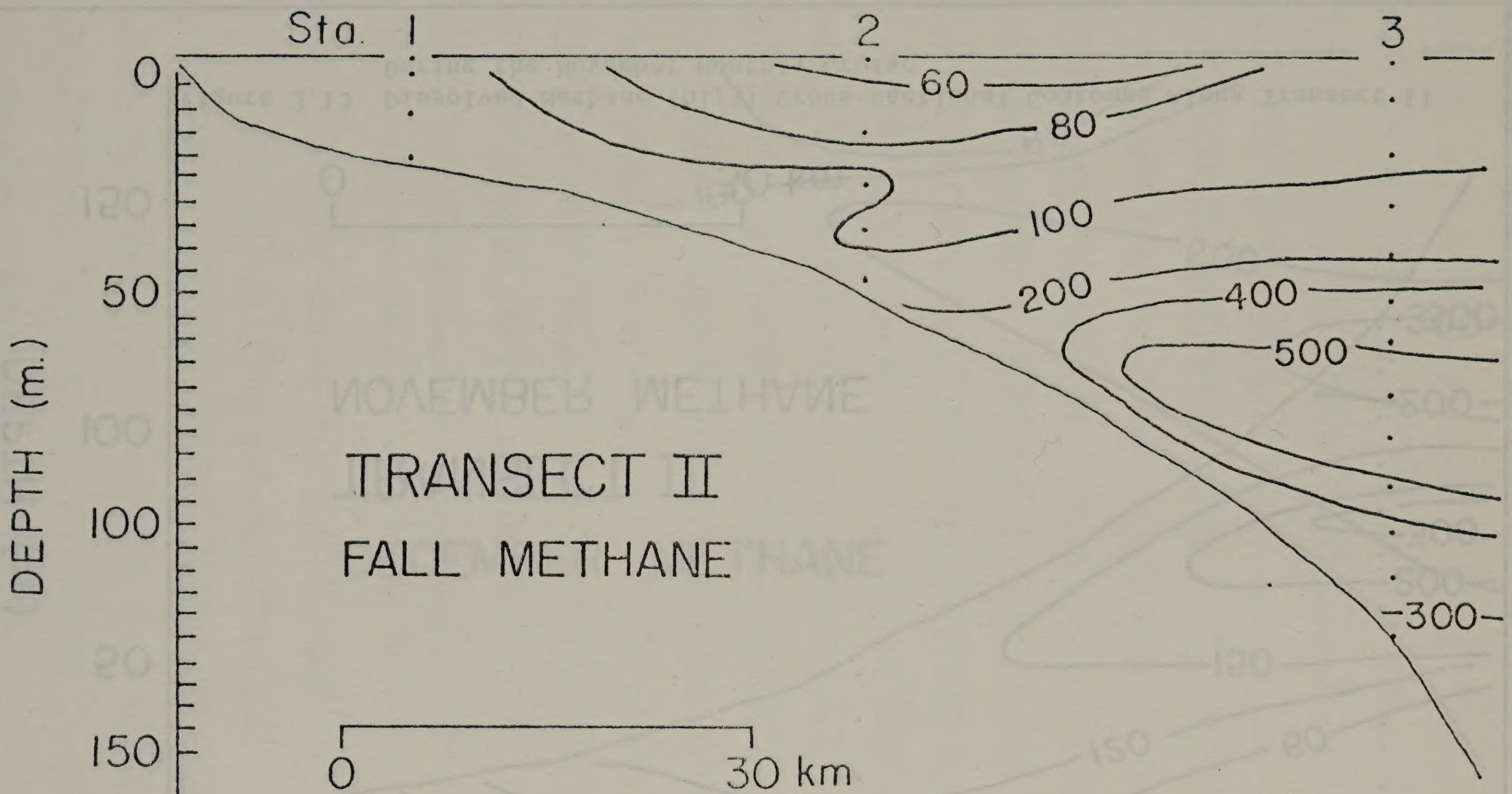

Figure 3.12 Dissolved Methane (nl/ℓ) Cross-sectional Contours Along Transect II During the Fall Seasonal Cruise.

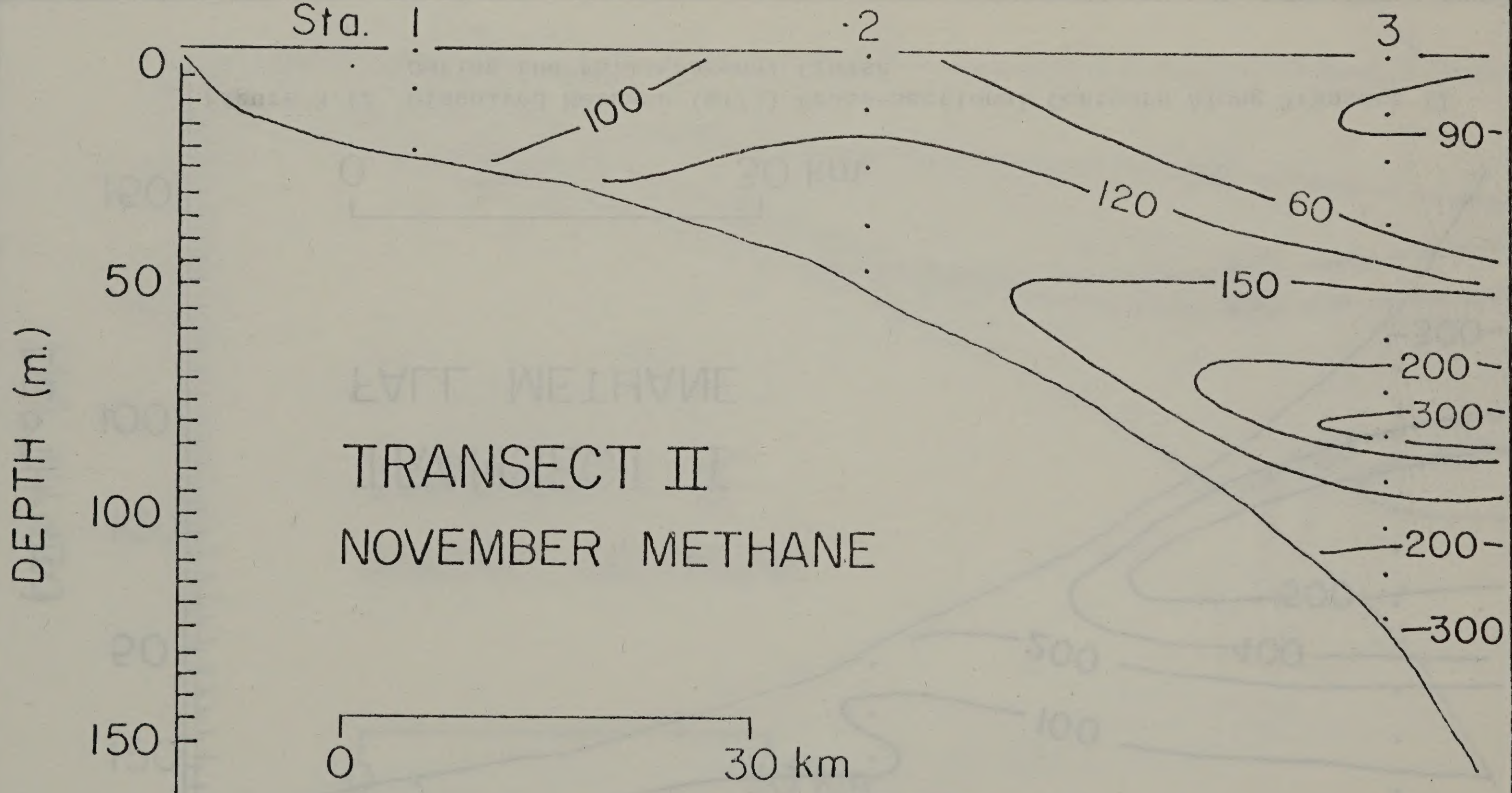

Figure 3.13 Dissolved Methane (nl/ℓ) Cross-sectional Contours Along Transect II During the November Monthly Cruise.

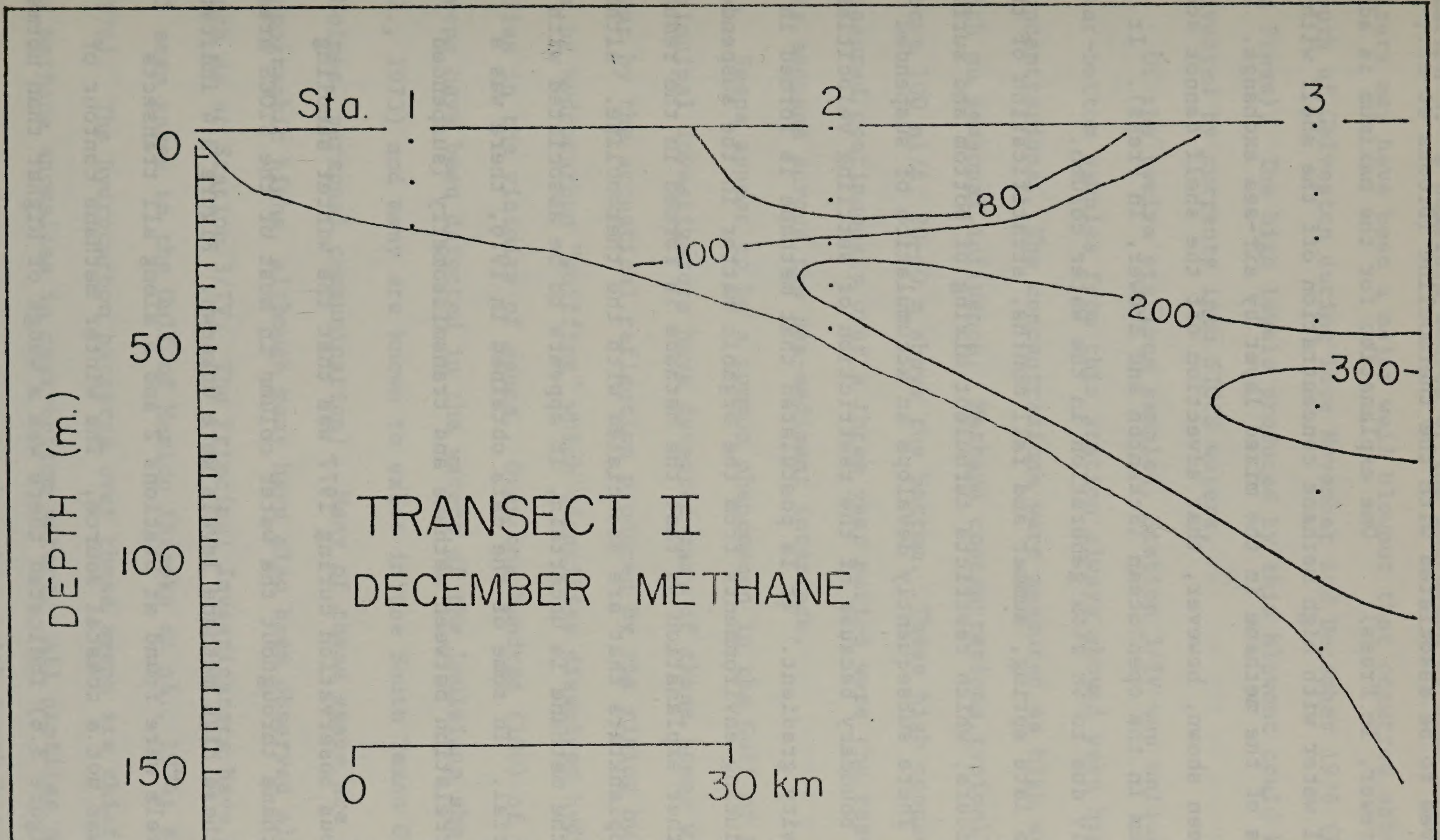

Figure 3.14 Dissolved Methane (nl/ℓ) Cross-sectional Contours Along Transect II During the December Monthly Cruise.

The origin of the mid-depth maximum is only partially understood. It has been observed to be associated with the thermocline (Brooks *et al.*, 1977, Scranton and Brewer, In Press). One explanation for the maximum is advection of coastal water with high methane concentration off the shelf with subsequent loss of the methane in the mixed layer by air-sea exchange. It has recently been shown, however, that advection off the shelf cannot account for this maximum in the open ocean (Scranton and Brewer, In Press). It is more probably due to *in situ* generation in the water column.

During the late spring, summer and fall months, stratification of the water column occurs, which restricts turbulent mixing of bottom and surface water masses. There subsequently develops an accumulation of suspended matter at this boundary because of the restriction of settling velocities across the density gradient. It is postulated that methane is formed in small micro-reducing environments from the organic matter in the suspended material. Another explanation is that the methane is formed in the reducing guts of zooplankters that are associated with the thermocline. Although the origin of the methane is uncertain, it appears to be associated with suspended material. In some of the data obtained in 1976, there was a good visual correlation between methane and transmissometry (suspended matter).

An anomalous observation during 1977 was that the winter sampling showed high methane throughout the water column in most of the STOCS area. The source of these high methane levels were unknown. Figure 3.4 indicates the highest levels were found at Stations 2 and 3 along all transects indicating it was not a coastal source. The winter methane contour of Transect II (Figure 3.6) indicated there was a wedge of higher than normal methane levels along the middle of this transect. The other transects

(Appendix B, Table 13) also showed this wedge of high methane extending past Station 3. A possible source of the intrusion of high methane into STOCS waters may have been a major well blowout that occurred about 100 miles south of Galveston during late November and December 1976 (Brooks *et al.*, In Press). The high levels produced by this blowout could have been advected by currents into STOCS waters.

Of the twelve stations sampled, Station 3/IV was unique in that the near-bottom samples from this station always showed very high methane concentrations. The concentrations were measured as high as 400 nl/ℓ, with no seasonal influence. Methane concentrations typically remained above 100 nl/ℓ, 20-40 m above the bottom. These high concentrations measured continuously over a three year period were the result of natural gas seepage across the sea-sediment interface.

Seeps appear to be a common phenomenon in the Gulf of Mexico. The Principal Investigators have detected many of these seeps with sonar equipment by the acoustical reflection produced by the rising bubble plume, or by hydrocarbon "sniffing" which identifies dissolved hydrocarbon anomalies in the vicinity of seeps. Over one hundred (100) of these bubbling seeps have been detected in the waters of the Louisiana shelf (Tinkle *et al.*, 1973) and many are known to exist in the South Texas OCS region (Holmes, personal communication). Many of these seeps are associated with topographic highs, although many have also been observed along flat bottom such as at Station 3/IV. The Principal Investigators have collected over 25 seep gases in the Gulf of Mexico (Brooks *et al.*, 1974; Bernard *et al.*, 1976). The hydrocarbon patterns over these seeps are chiefly characterized by methane anomalies in near-bottom waters. All profiles over seeps show a significant increase in dissolved methane with depth because of increasing solution of methane due to increased partial pressure in the bubbles with depth.

Areal Distribution of Olefins

The concentrations of unsaturated hydrocarbons (*e.g.* ethene and propene) were measured in surface, half the depth of the photic zone, and near-bottom samples at the 12 line stations. Ethene was also measured at some additional intermediate depths. The number of observations, mean, minimum, and maximum values for the olefins are shown in Table 3.4. The unsaturates dominated over their saturated analogs in most areas of the STOCS, with exceptions generally occurring in the deeper waters, or Station 3 areas, along the four transects. Ethene averaged 4.8 nl/ℓ in the STOCS area, but concentrations as high as 20 nl/ℓ were measured. Propene concentrations were almost always a factor of four lower than ethene concentrations, only averaging 1.0 nl/ℓ in the STOCS area in 1977.

Unlike methane, the exchange of olefins across the air-sea interface in the STOCS region is more speculative. This is because there are no well-established atmospheric partial pressures for ethene and propene. Also, solubility data at oceanic temperatures and salinities do not exist in the literature. However, since oxidation of the olefins occurs within hours in the atmosphere, all the olefins found in the surface waters of the Gulf must be biologically derived and/or introduced through losses from refined products during transportation or manufacturing operations. It is therefore assumed that the direction of olefin exchange across the air-sea interface is from the ocean to the atmosphere.

Figures 3.15 and 3.16 show near-surface concentrations of ethene and propene, respectively, during the seasonal cruises in the STOCS area in 1977. These figures indicated that the highest concentrations of olefins occurred at inshore stations during the winter seasonal cruise. The oppo-

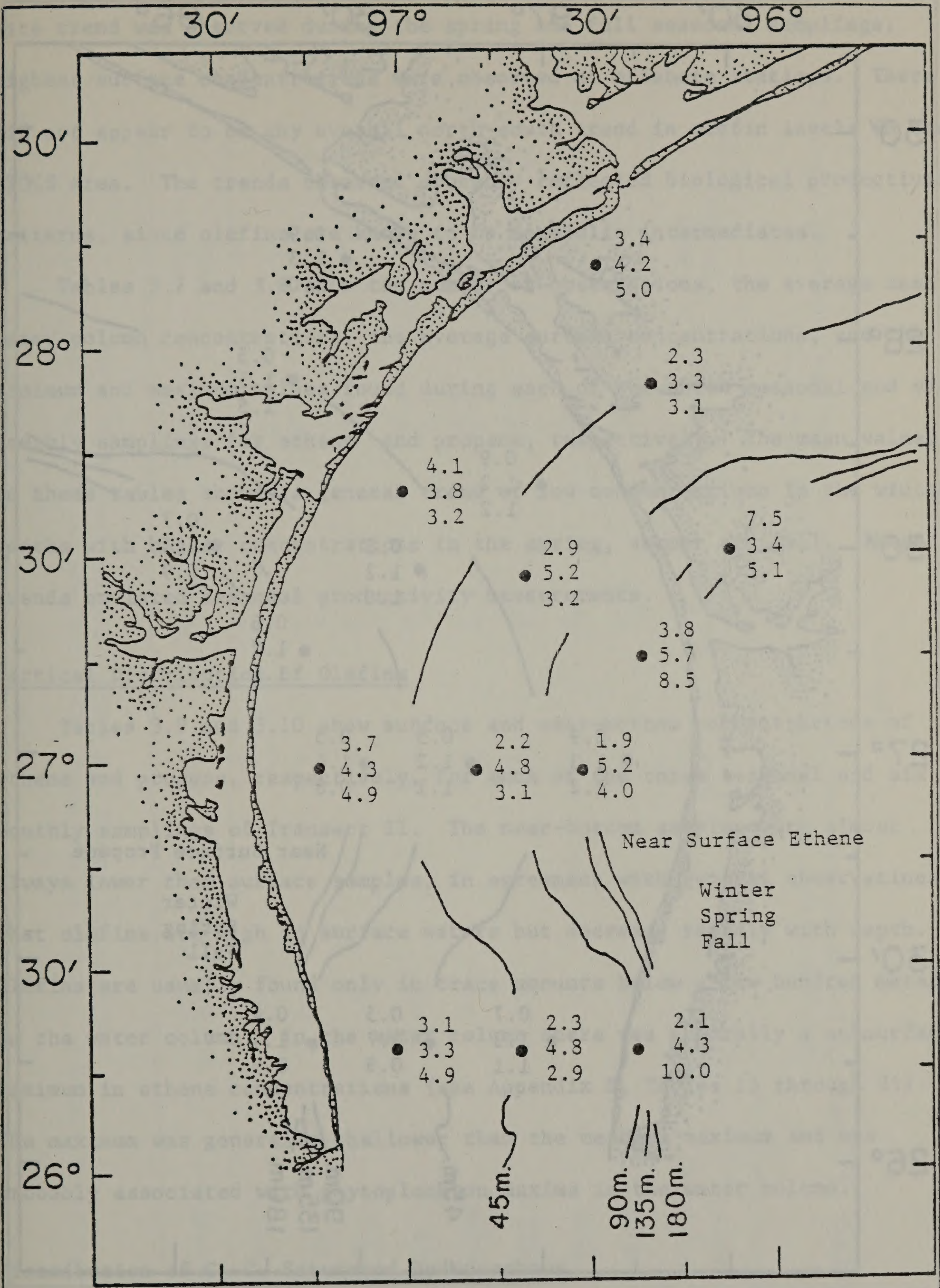

Figure 3.15 Near-Surface Ethene Concentrations (nl/ℓ) in the STOCS Area During the Seasonal Cruises in 1977.

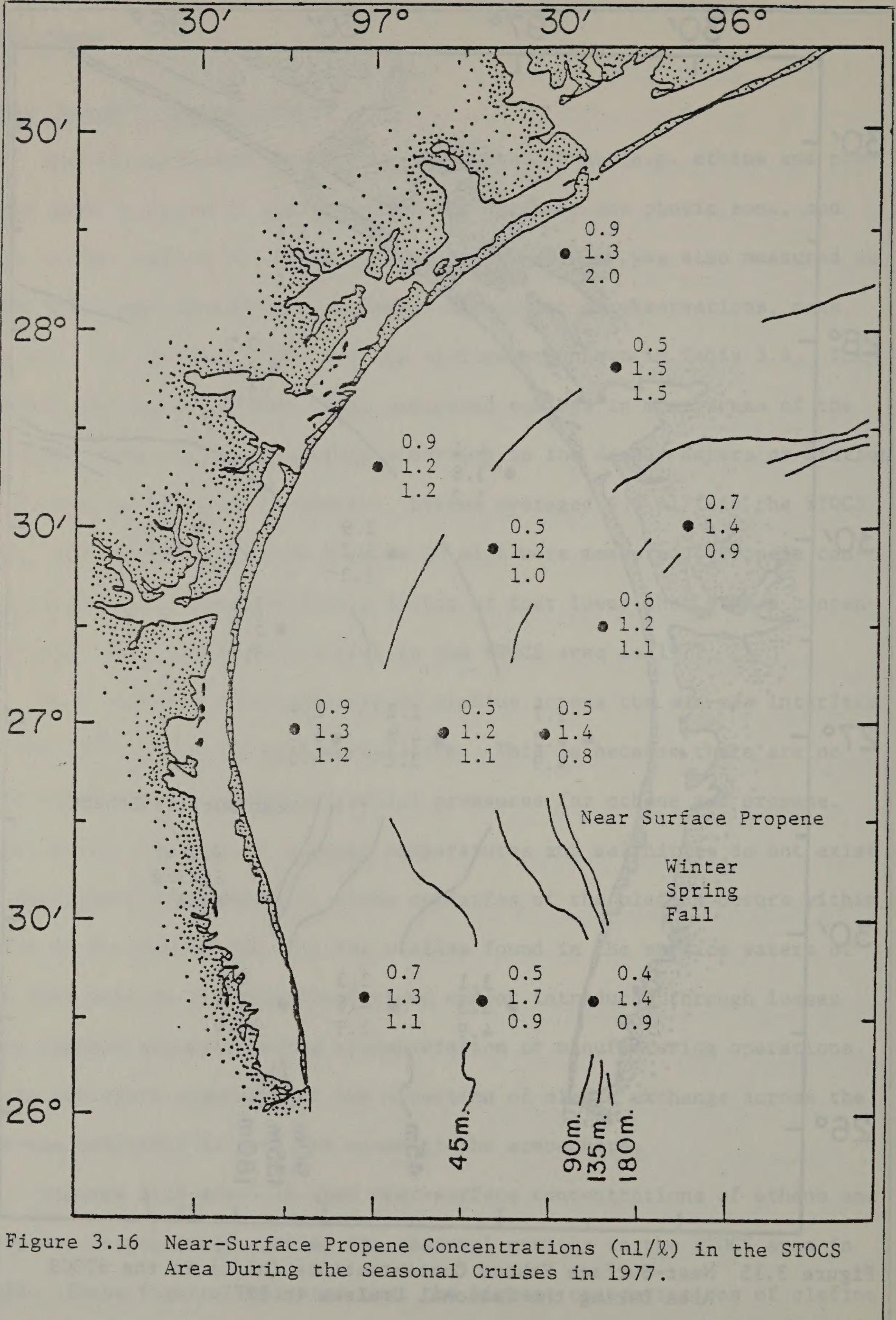

Figure 3.16 Near-Surface Propene Concentrations (nl/ℓ) in the STOCS Area During the Seasonal Cruises in 1977.

site trend was observed during the spring and fall seasonal samplings; highest surface concentrations were observed at offshore stations. There did not appear to be any overall north-south trend in olefin levels in the STOCS area. The trends observed probably reflected biological productivity patterns, since olefins are known to be metabolic intermediates.

Tables 3.7 and 3.8 show the number of observations, the average mean water column concentrations, the average surface concentrations, and the minimum and maximum values found during each of the three seasonal and six monthly samplings for ethene and propene, respectively. The mean values in these tables showed a general trend of low concentrations in the winter months with higher concentrations in the spring, summer and fall. These trends emulated seasonal productivity measurements.

Vertical Distribution of Olefins

Tables 3.9 and 3.10 show surface and near-bottom concentrations of ethene and propene, respectively, for each of the three seasonal and six monthly samplings of Transect II. The near-bottom samples were almost always lower than surface samples, in agreement with general observations that olefins are high in surface waters but decrease rapidly with depth. Olefins are usually found only in trace amounts below a few hundred meters in the water column. In the water column there was generally a subsurface maximum in ethene concentrations (see Appendix B, Tables 13 through 24). The maximum was generally shallower than the methane maximum and was probably associated with phytoplankton maxima in the water column.

Distribution of C_2-C_4 Saturated Hydrocarbons

Ethane and propane concentrations were measured at the 12 line stations. The number of observations, mean, minimum, and maximum values for these sat-

TABLE 3.7

SUMMARY OF NUMBER OF ETHENE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (in nl/ℓ) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1977).

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	59	2.6	0.11	7.5	3.3
March	9	3.7	2.7	5.0	4.0
April	12	3.5	1.8	4.6	3.5
Spring	61	4.6	1.0	9.8	4.4
July	22	4.7	0.7	14.0	4.8
August	19	7.0	1.3	20.0	4.9
Fall	86	5.6	0.8	20.5	4.8
November	19	4.3	1.7	8.4	4.4
December	17	3.4	7.2	4.8	3.8

TABLE 3.8

SUMMARY OF NUMBER OF PROPENE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (in nl/ℓ) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1977).

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	36	0.6	0.3	1.0	0.6
March	9	1.2	0.6	1.7	1.4
April	9	1.3	0.6	1.9	1.8
Spring	36	1.1	0.4	1.9	1.3
July	12	1.1	0.3	1.8	1.2
August	16	1.5	0.5	2.6	1.5
Fall	36	0.9	0.3	2.0	1.1
November	9	1.1	0.5	2.6	1.3
December	9	1.2	0.4	1.7	1.3

TABLE 3.9

SURFACE AND NEAR-BOTTOM ETHENE CONCENTRATIONS (nl/ℓ) AT STOCS STATIONS ALONG TRANSECT II.

SAMPLING MONTH	STATION 1/II	2/II	3/II
Surface			
Winter	4.1	2.9	3.8
March	4.1	3.6	4.4
April	4.1	3.2	3.1
Spring	3.8	5.2	5.7
July	3.3	6.0	5.2
August	3.3	5.9	5.6
Fall	3.2	3.2	8.5
November	2.2	6.2	4.8
December	4.4	3.5	3.4
Near-Bottom			
Winter	4.0	2.9	1.2
March	3.8	3.5	2.7
April	4.3	3.2	1.8
Spring	4.2	3.8	1.0
July	4.9	1.6	0.7
August	6.1	20.4	1.3
Fall	3.3	6.5	1.7
November	2.5	2.9	1.7
December	3.6	4.3	1.5

TABLE 3.10

SURFACE AND NEAR-BOTTOM PROPENE CONCENTRATIONS (nl/ℓ) AT STOCS STATIONS ALONG TRANSECT II.

SAMPLING MONTH	STATION 1/II	2/II	3/II
	Surface		
Winter	0.9	0.5	0.6
March	1.7	1.5	1.0
April	1.9	1.5	1.9
Spring	1.2	1.2	1.2
July	0.6	1.3	1.8
August	1.4	1.5	1.7
Fall	1.2	1.0	1.1
November	0.5	2.6	0.7
December	1.7	1.3	1.0
	Near-Bottom		
Winter	1.0	0.6	0.4
March	1.3	0.8	0.6
April	1.4	1.0	0.6
Spring	1.3	0.7	0.4
July	0.7	0.8	0.3
August	1.2	1.4	0.5
Fall	1.1	0.8	0.4
November	0.9	1.2	0.6
December	1.2	1.1	0.3

urated compounds are shown in Table 3.4. The saturated hydrocarbon concentrations were generally lower than corresponding olefins in the coastal waters of the STOCS area. Ethane and propane showed mean values of 1.7 and 0.9, respectively, nl/ℓ in the STOCS area. Figures 3.17 and 3.18 show surface ethane and propane concentrations, respectively, during the seasonal cruises in the STOCS area in 1977. Concentrations of these LMWH components were generally higher at inshore stations and decreased seaward. They showed little seasonal variation as seen by Tables 3.11 and 3.12, which are monthly tabulations of numbers of observations, mean, minimum and maximum concentrations for ethane and propane. Butane levels were almost always below detection limits in the STOCS area.

The saturated C_2-C_4 hydrocarbons have man-derived and natural sources similar to methane. They are derived from petroleum, either from offshore platforms, transportation activities, or runoff. Although there is no evidence that they are produced *in situ* in the water column, they are found in trace amounts in gas seepage. Most biogenic gas contains small amounts ($< 0.5\%$) of ethane and parts per million quantities of propane. It might be reasonable to assume that some of the long-lived anthropogenic hydrocarbons (*e.g.*, ethane and propane) found in marine atmospheres are deposited into the ocean either by rainout or by air-sea exchange.

Tables 3.13 and 3.14 show surface and near-bottom ethane and propane concentrations along Transect II. There was no large variation in the vertical distribution of these components with depth, although bottom samples were generally higher than surface samples, probably reflecting diffusion or seepage out of the sediments.

Hydrocarbon Correlations

As mentioned in the earlier discussions, several LMWH showed close

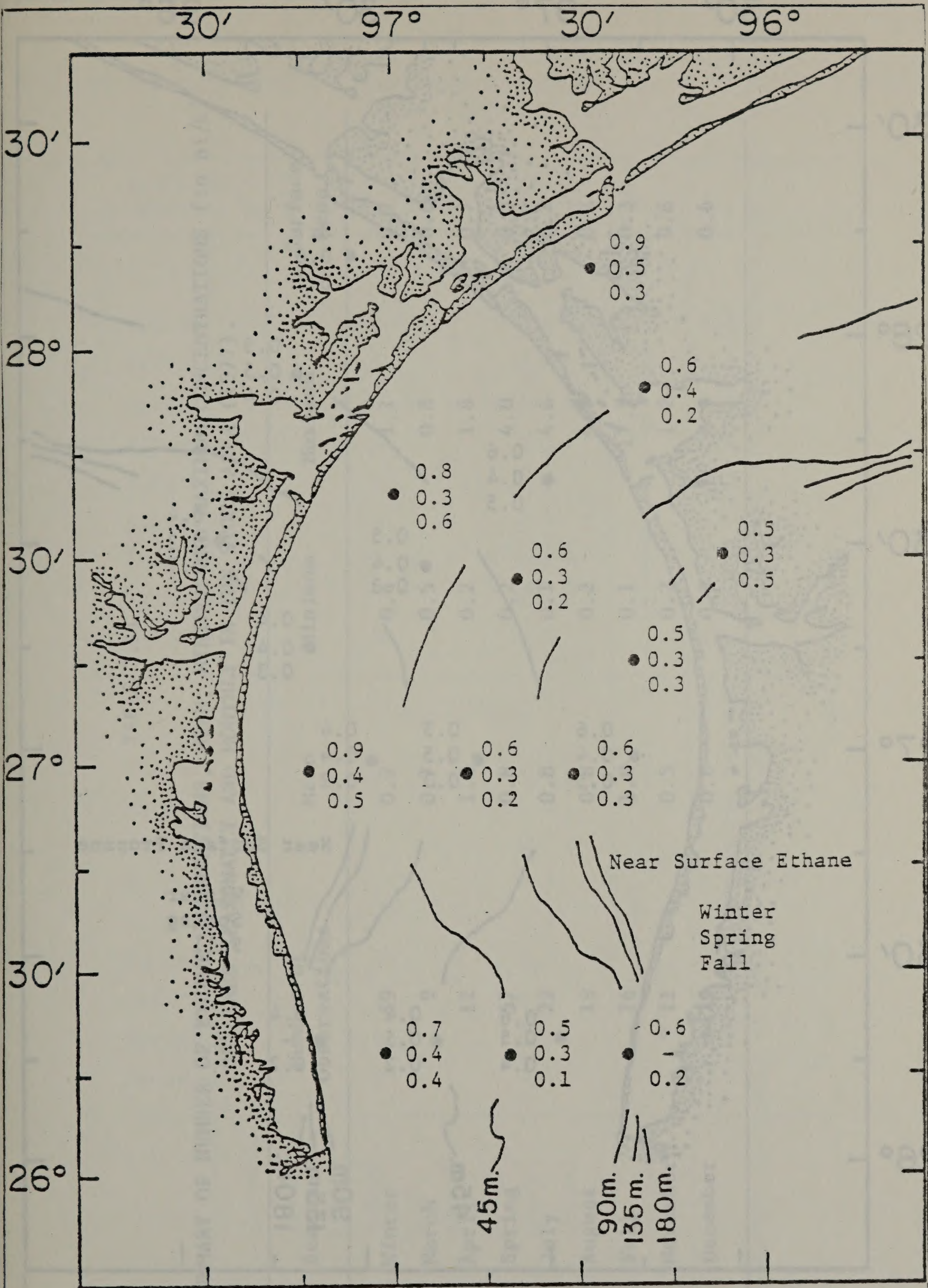

Figure 3.17 Near-Surface Ethane Concentrations (nl/ℓ) in the STOCS Area During the Seasonal Cruises in 1977.

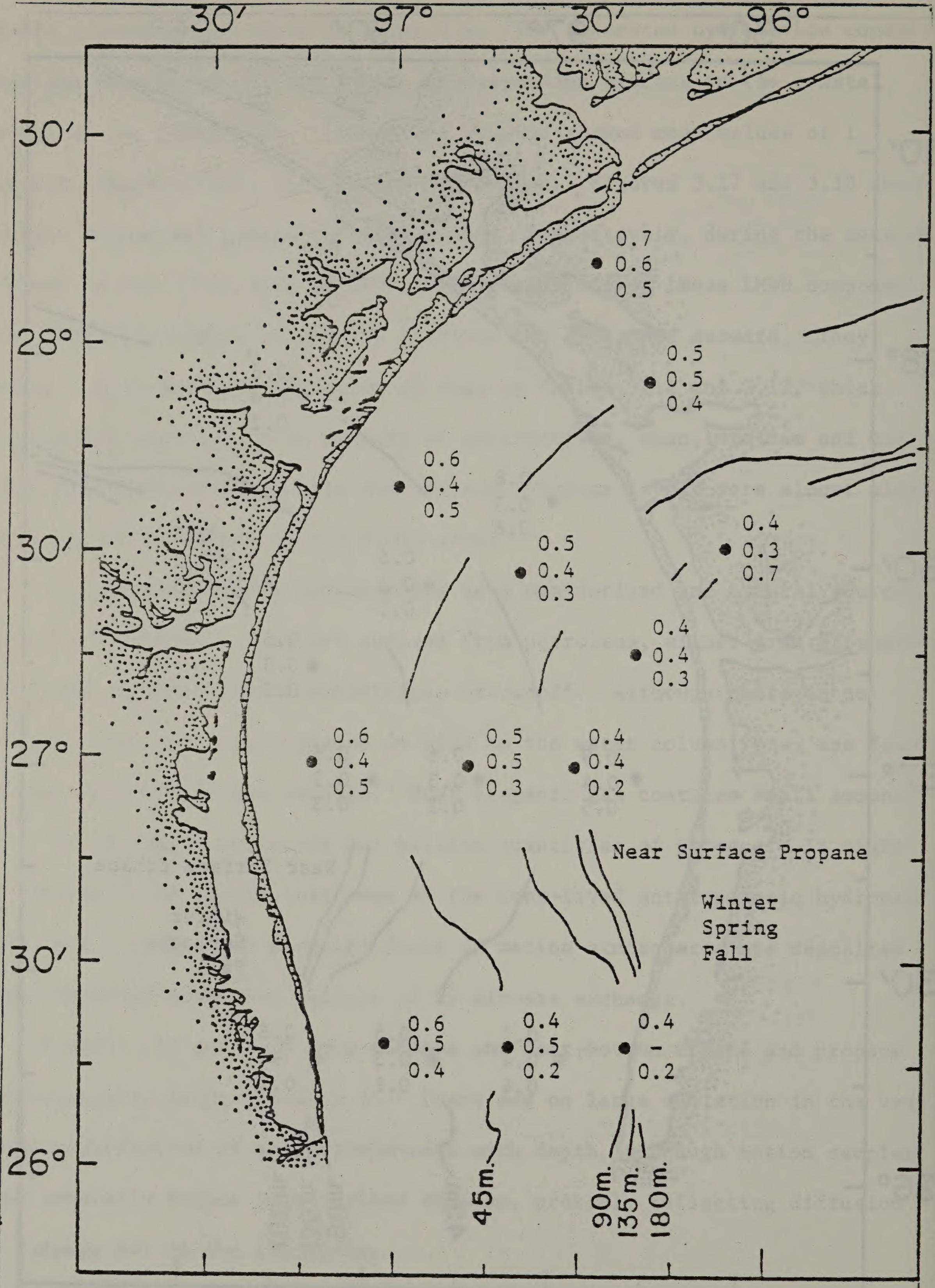

Figure 3.18 Near-Surface Propane Concentrations (nl/ℓ) in the STOCS Area During the Seasonal Cruises in 1977.

TABLE 3.11

SUMMARY OF NUMBER OF ETHANE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (in nl/ℓ) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1977).

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	59	0.7	0.4	1.1	0.7
March	9	0.7	0.5	0.8	0.6
April	12	1.0	0.2	1.8	0.7
Spring	57	0.8	0.2	4.0	0.3
July	22	0.8	0.1	4.6	0.2
August	19	0.8	0.2	4.6	0.2
Fall	76	1.0	0.1	17.5	0.3
November	11	0.5	0.2	1.2	0.6
December	9	0.6	0.3	0.9	0.6

TABLE 3.12

SUMMARY OF NUMBER OF PROPANE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (in nl/ℓ) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1977).

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	36	0.5	0.4	0.7	0.5
March	9	0.5	0.4	0.7	0.5
April	9	0.7	0.3	1.2	0.7
Spring	43	0.6	0.3	1.5	0.4
July	9	0.5	0.3	1.1	0.3
August	11	0.9	0.3	4.5	0.6
Fall	36	0.4	0.2	2.2	0.4
November	9	0.3	0.2	0.7	0.3
December	9	0.4	0.3	0.5	0.4

TABLE 3.13

SURFACE AND NEAR-BOTTOM ETHANE CONCENTRATIONS (nl/ℓ) AT STOCS STATIONS ALONG TRANSECT II.

SAMPLING MONTH	STATION 1/II	2/II	3/II
Surface			
Winter	0.8	0.6	0.5
March	0.8	0.6	0.5
April	1.6	0.4	0.2
Spring	0.3	0.3	0.3
July	0.1	0.2	0.1
August	0.2	0.2	0.3
Fall	0.3	0.2	0.5
November	1.2	0.4	0.2
December	0.9	0.8	0.3
Near-Bottom			
Winter	0.8	0.7	0.4
March	0.7	0.8	0.8
April	1.7	1.3	1.0
Spring	0.6	1.0	0.9
July	0.9	0.3	0.3
August	0.6	0.9	0.5
Fall	0.3	0.8	0.4
November	0.4	0.4	0.9
December	0.9	0.3	0.3

TABLE 3.14

SURFACE AND NEAR-BOTTOM PROPANE CONCENTRATIONS (nl/ℓ) AT STOCS STATIONS ALONG TRANSECT II.

SAMPLING MONTH	STATION 1/II	 2/II	 3/II
	Surface		
Winter	0.6	0.5	0.4
March	0.6	0.6	0.4
April	1.1	0.5	0.4
Spring	0.4	0.4	0.4
July	0.3	0.3	0.3
August	0.3	0.3	1.1
Fall	0.5	0.4	0.7
November	0.4	0.3	0.3
December	0.4	0.3	0.3
	Near-Bottom		
Winter	0.5	0.5	0.5
March	0.7	0.5	0.5
April	0.9	0.5	1.2
Spring	0.7	1.0	1.0
July	0.9	1.1	0.4
August	0.7	0.5	0.7
Fall	0.4	0.6	0.5
November	0.3	0.3	0.7
December	0.4	0.3	0.5

correlations with other chemical and biological parameters. Table 3.15 shows correlations of the LMWH with each other and other selected chemical parameters measured in our study. Methane showed little correlation with other LMWH. There was a correlation between methane and ethane and no correlation between methane and propane. There was a correlation coefficient of 0.74 between ethane and propane. Although both ethene and propene are products of metabolic activities, they showed only a weak correlation coefficient of 0.45.

Dissolved Oxygen

All dissolved oxygen measurements performed in 1977 are tabulated in Appendix B, Tables 1-12. Mean monthly oxygen concentrations, as well as minimum and maximum values observed in the STOCS area in 1977, are reported in Table 3.16. Table 3.17 lists surface and near-bottom dissolved oxygen values measured seasonally and monthly at the three primary stations on Transect II. The surface concentrations at all stations were highest in the winter months and decreased in the summer, reflecting seasonal changes in surface temperature and salinities.

Near-surface temperatures and salinities are plotted for the winter, spring, and fall on Figures 3.19 and 3.20, respectively. Wide temperature variations were apparent between the seasons and between nearshore and offshore stations of a particular season. Likewise, salinity was affected by proximity to the land. Increased runoff in the spring drastically reduced salinity at nearshore stations.

Gas solubility in seawater increases with decreasing water temperatures and salinity so surface oxygen concentrations are seasonally controlled, as further illustrated by surface measurements from the 12 primary stations shown in Figure 3.21. Not only were surface oxygen concen-

TABLE 3.15

CORRELATION COEFFICIENTS[†] ON STOCS 1977 DATA.

Variable	Methane	Ethene	Ethane	Propene	Propane
Methane	1.0	-	0.611	-	-
Ethene		1.0	-	0.450	-
Ethane	0.611	-	1.0	-	0.741
Propene	-	0.450	-	1.0	-
Propane	-	-	0.741	-	1.0
Depth	-	-	-	0.520	-
Temperature	-		-	0.514	-
Salinity	-	-	-	-	-
Oxygen	-	-	-	-	-
Phosphate	-	-	-	-	-
Nitrate	-	-	-	0.424	-
Silicate	-	-	-	-	-

[†] Only correlations greater than 0.400 are tabulated.

TABLE 3.16

SUMMARY OF NUMBER OF OXYGEN OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (in ml/ℓ) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1977).

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	82	5.53	4.19	6.95	5.73
March	18	5.46	4.46	6.00	5.62
April	25	5.03	4.55	5.37	5.11
Spring	97	4.96	3.77	5.43	5.06
July	32	4.73	3.03	5.70	4.81
August	31	4.79	3.30	5.48	4.64
Fall	102	4.53	3.37	5.33	4.57
November	29	4.65	3.57	5.21	4.74
December	27	4.65	3.34	5.40	5.07

TABLE 3.17

SURFACE AND NEAR-BOTTOM DISSOLVED OXYGEN CONCENTRATIONS (ml/ℓ) AT STOCS STATIONS ALONG TRANSECT II.

SAMPLING MONTH	STATIONS 1/II	2/II	3/II
		Surface	
Winter	6.06	5.92	5.55
March	6.00	5.64	5.67
April	4.90	5.16	5.05
Spring	4.88	5.04	5.00
July	4.83	4.79	4.83
August	4.73	4.74	4.55
Fall	4.51	4.12	4.57
November	5.08	4.26	4.74
December	5.40	5.05	4.87
		Near-Bottom	
Winter	5.33	5.83	4.19
March	5.70	5.34	4.46
April	4.70	5.25	5.11
Spring	4.18	4.88	4.59
July	4.22	5.14	3.02
August	5.19	5.38	3.30
Fall	3.91	4.32	3.84
November	4.65	4.71	3.57
December	5.12	4.84	3.34

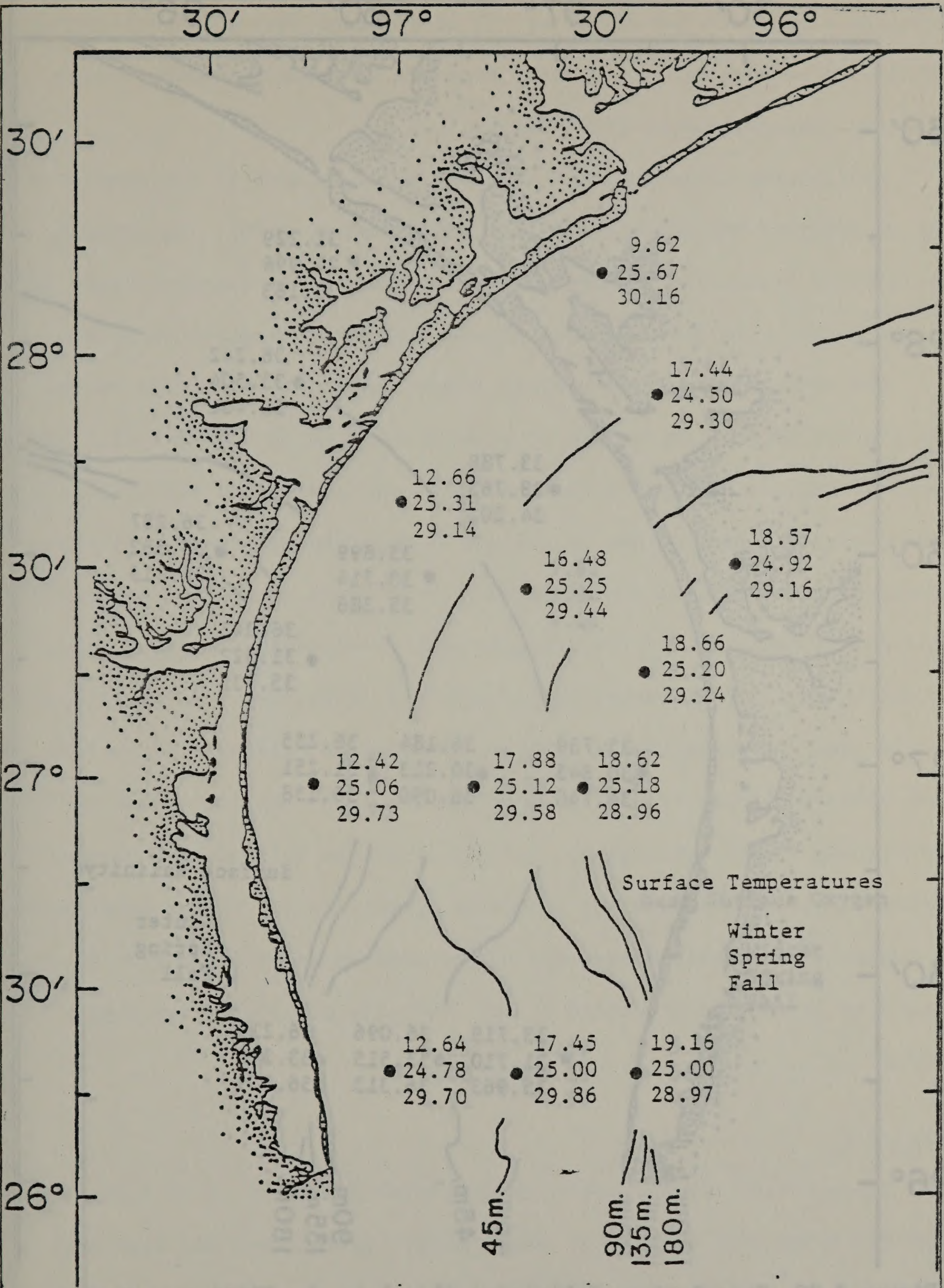

Figure 3.19 Near-Surface Temperatures (°C) in the STOCS Area During the Seasonal Cruises in 1977.

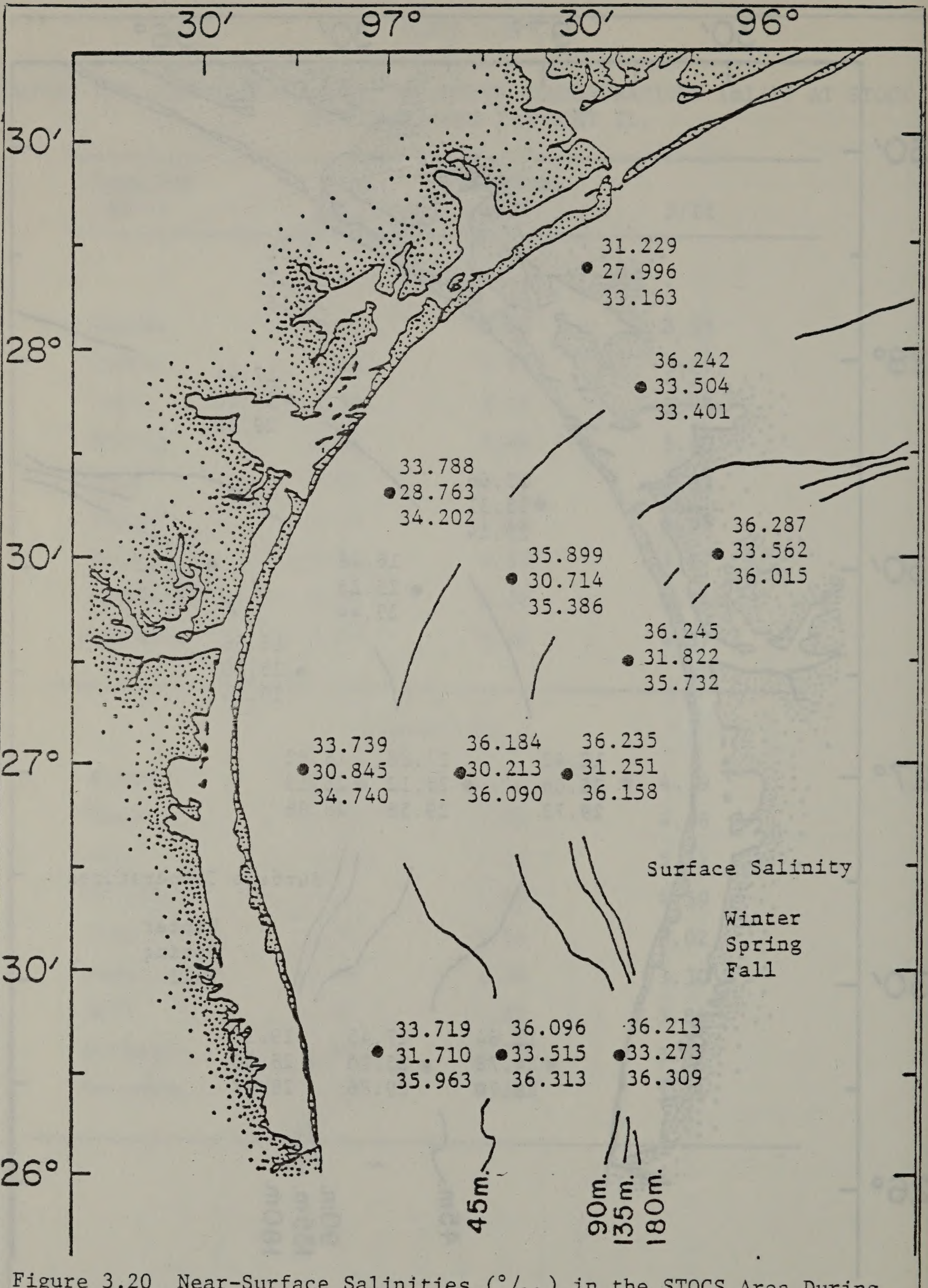

Figure 3.20 Near-Surface Salinities (°/₀₀) in the STOCS Area During the Seasonal Cruises in 1977.

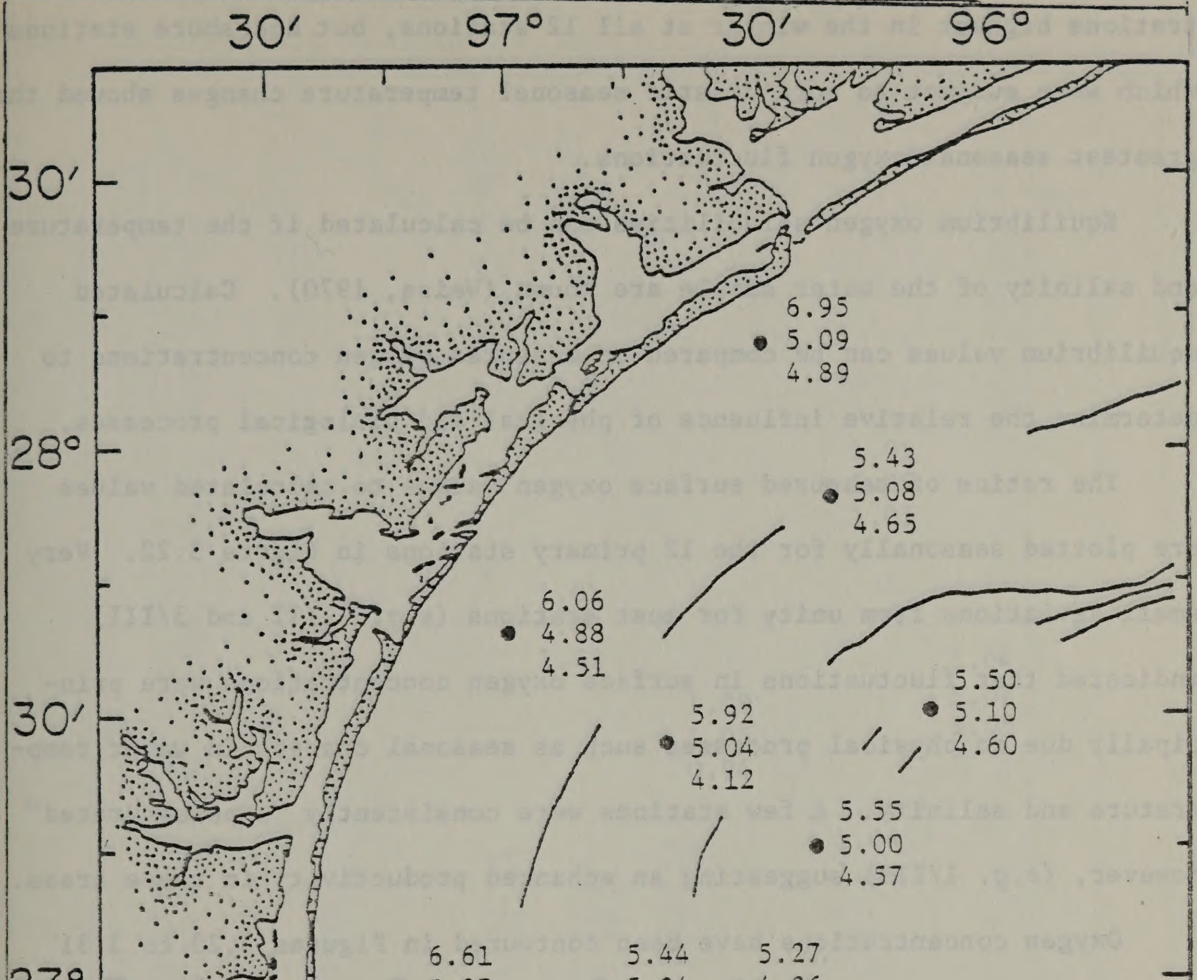

Figure 3.21 Near-Surface Dissolved Oxygen Concentrations (ml/ℓ) in the STOCS Area During the Seasonal Cruises in 1977.

trations highest in the winter at all 12 stations, but nearshore stations which were subject to more drastic seasonal temperature changes showed the greatest seasonal oxygen fluctuations.

Equilibrium oxygen solubilities can be calculated if the temperature and salinity of the water sample are known (Weiss, 1970). Calculated equilibrium values can be compared to measured oxygen concentrations to determine the relative influence of physical and biological processes.

The ratios of measured surface oxygen values to calculated values are plotted seasonally for the 12 primary stations in Figure 3.22. Very small deviations from unity for most stations (*e.g.* 1/II and 3/III) indicated that fluctuations in surface oxygen concentrations were principally due to physical processes such as seasonal changes in water temperature and salinity. A few stations were consistently "supersaturated" however, (*e.g.* 1/III) suggesting an enhanced productivity in these areas.

Oxygen concentrations have been contoured in Figures 3.23 to 3.31 with cross-sectional maps of Transect II for each sampling period of 1977. The figures showed the seasonal variations of the intrusion of oxygen-depleted bottom water and the increased stratification of the water column in summer. The highly-oxygenated winter surface water could also be traced as it was formed nearshore and gradually displaced from winter to summer.

Nutrients

All nutrient concentration measurements performed in 1977 are reported along with respective temperatures, salinities, and dissolved oxygen concentrations in Appendix B, Tables 1-12. Nutrient concentrations are reported in micromolar (μM) units which are equivalent to previously used microgram-atom units. The nutrients, nitrate, phosphate, and sili-

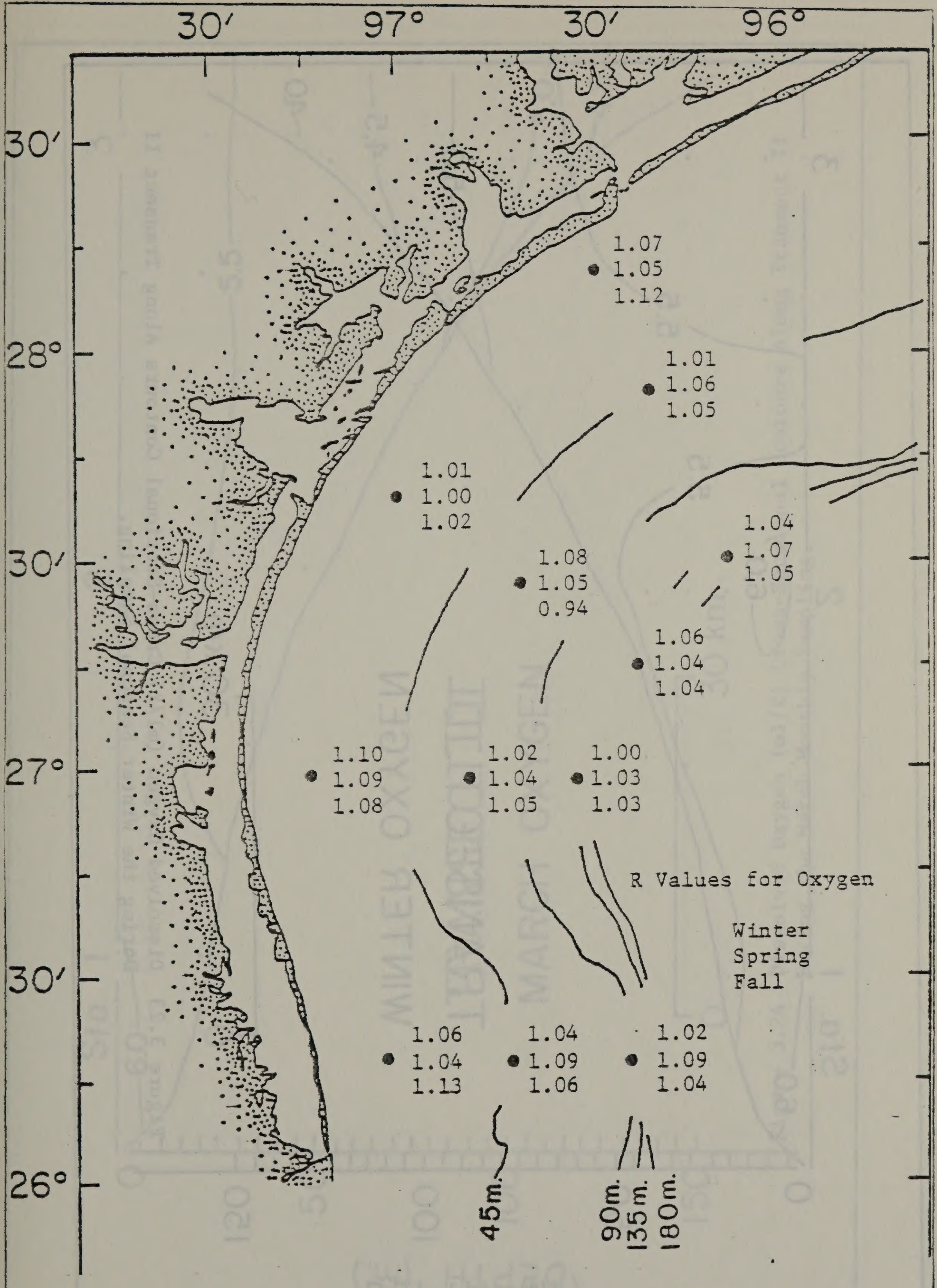

Figure 3.22 Near-Surface R (Degree of Saturation) Values for Dissolved Oxygen in the STOCS Area During the Seasonal Cruises.

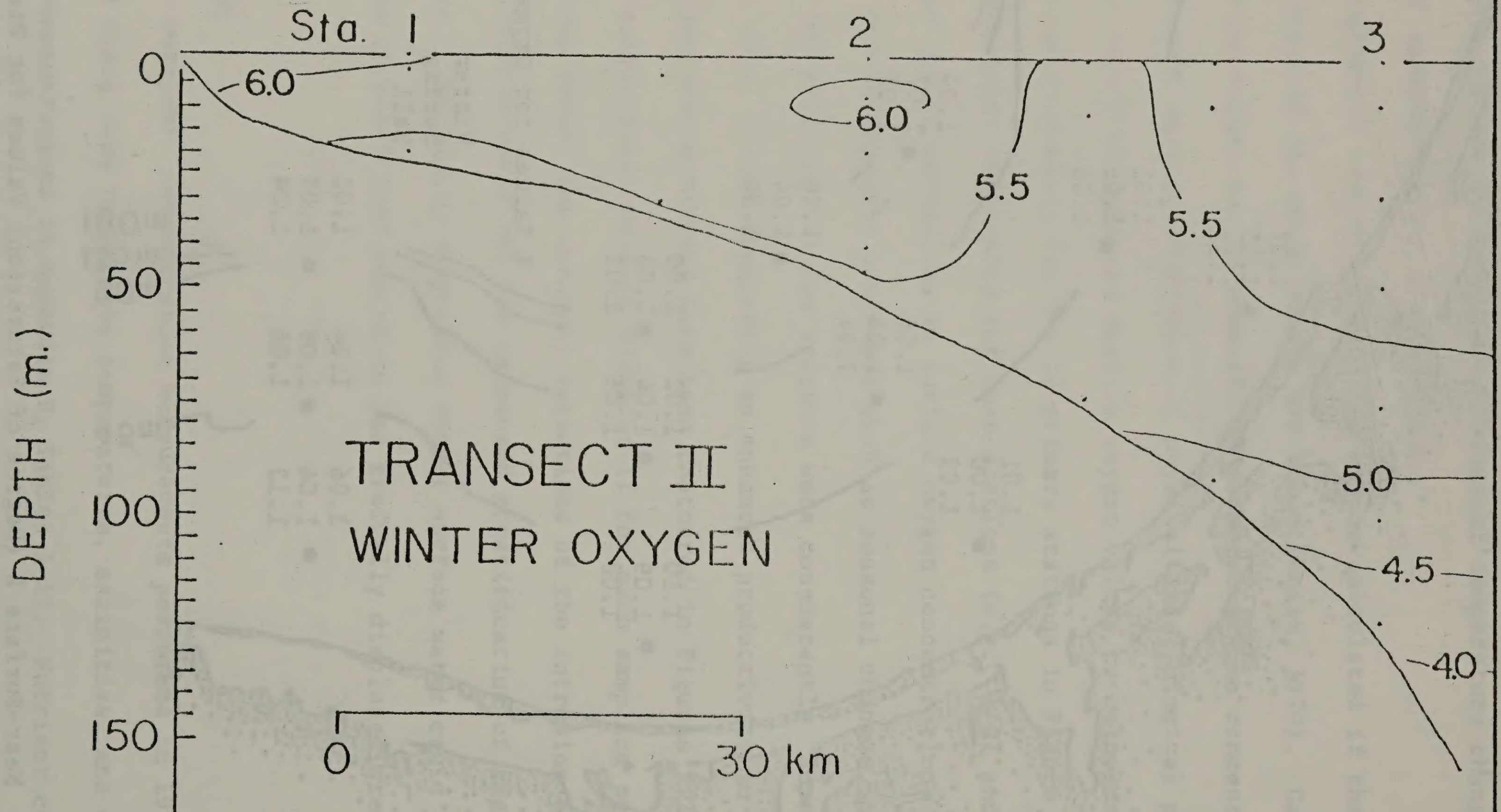

Figure 3.23 Dissolved Oxygen (ml/ℓ) Cross-Sectional Contours Along Transect II During the Winter Seasonal Sampling.

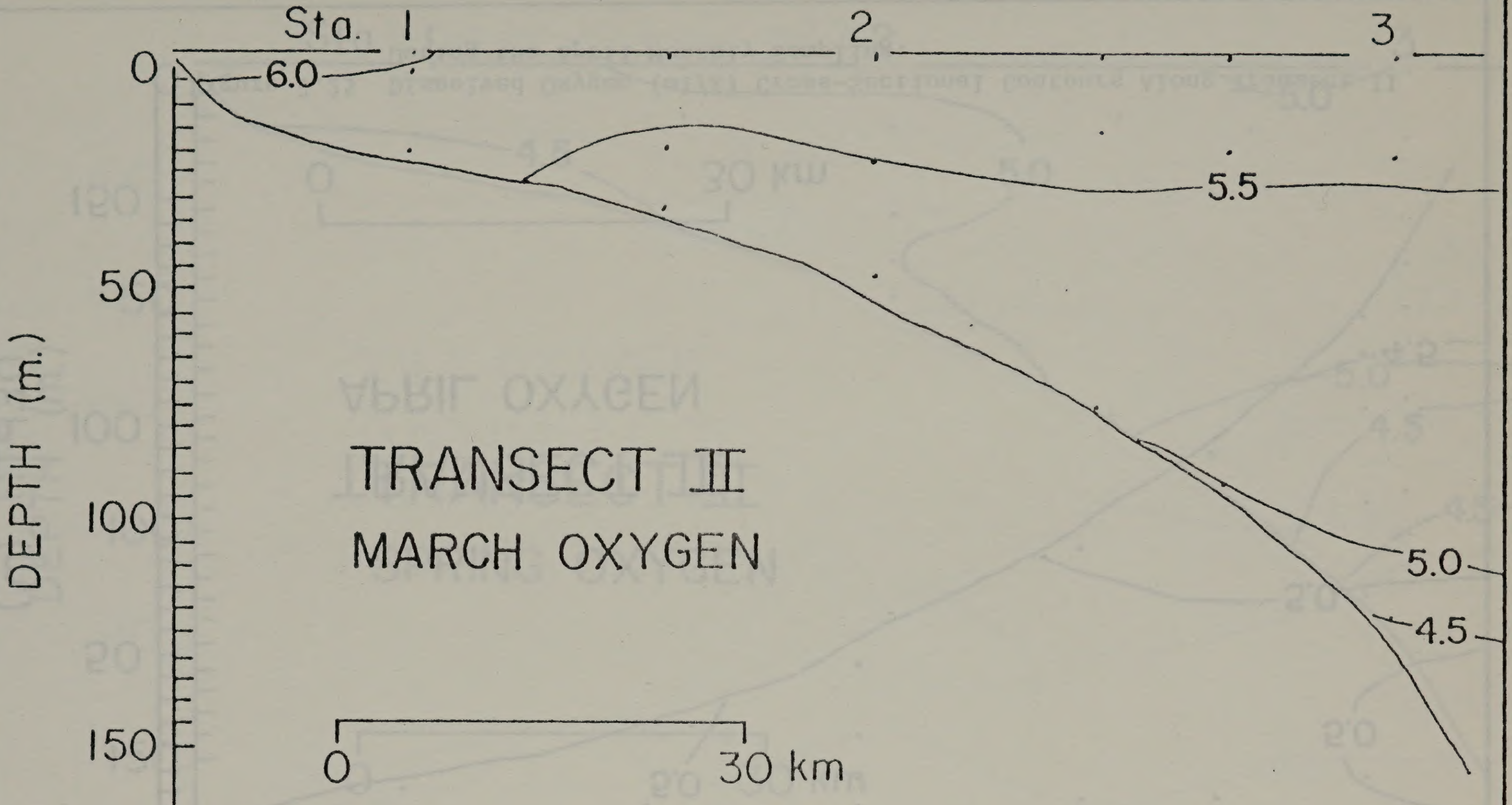

Figure 3.24 Dissolved Oxygen (ml/ℓ) Cross-Sectional Contours Along Transect II During the March Monthly Sampling.

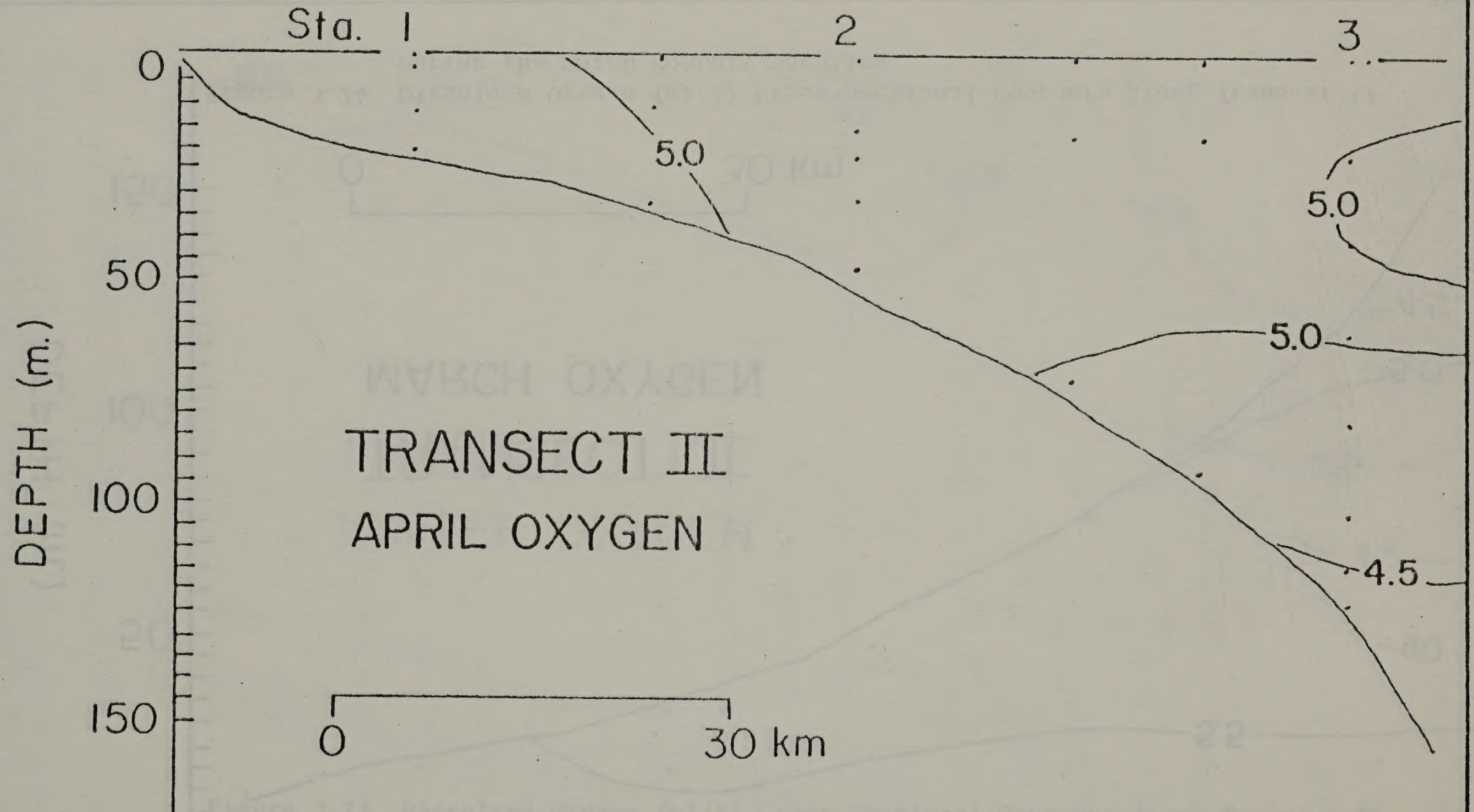

Figure 3.25 Dissolved Oxygen (ml/ℓ) Cross-Sectional Contours Along Transect II During the April Monthly Sampling.

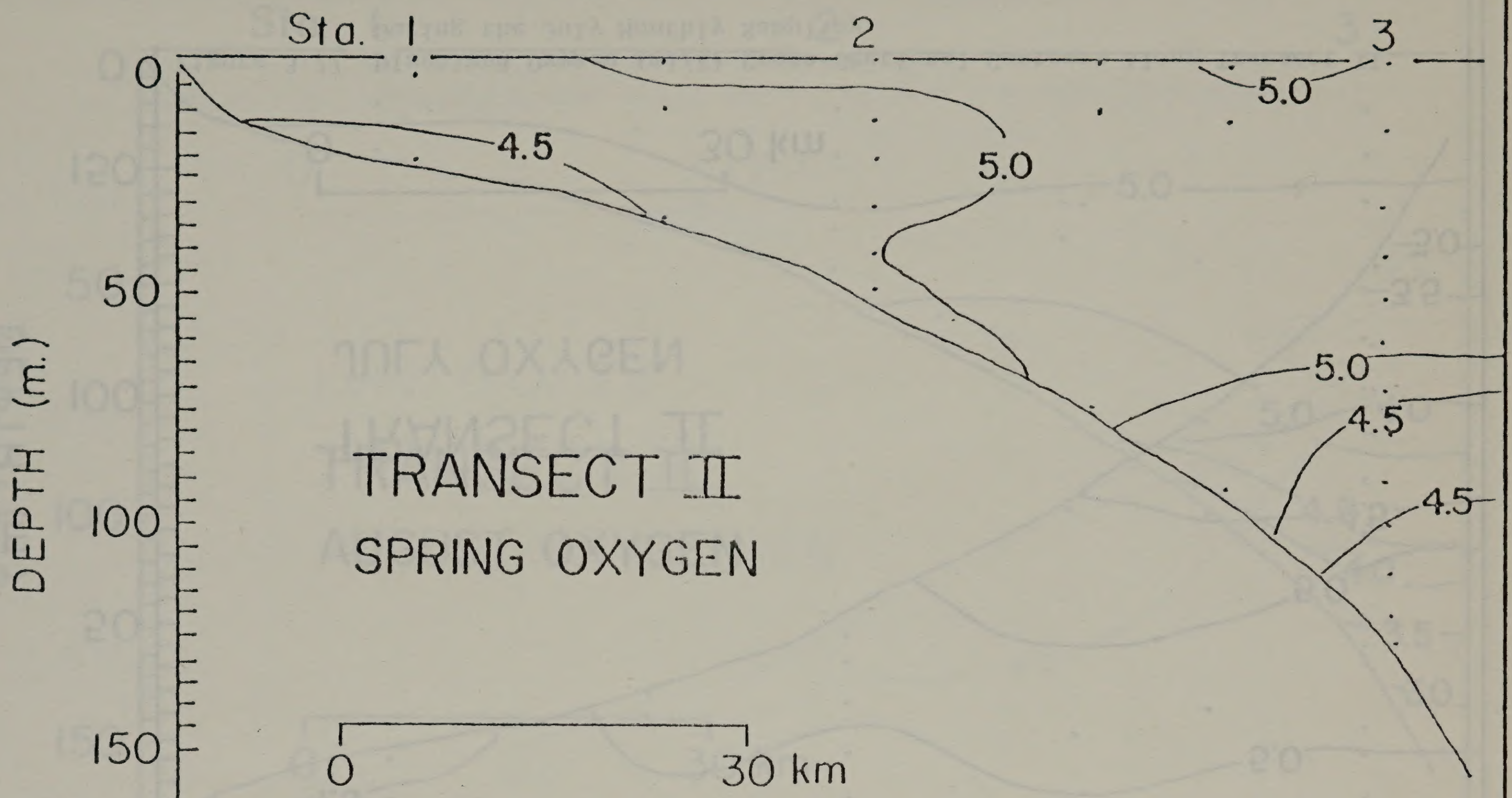

Figure 3.26 Dissolved Oxygen (ml/ℓ) Cross-Sectional Contours Along Transect II During the Spring Seasonal Sampling.

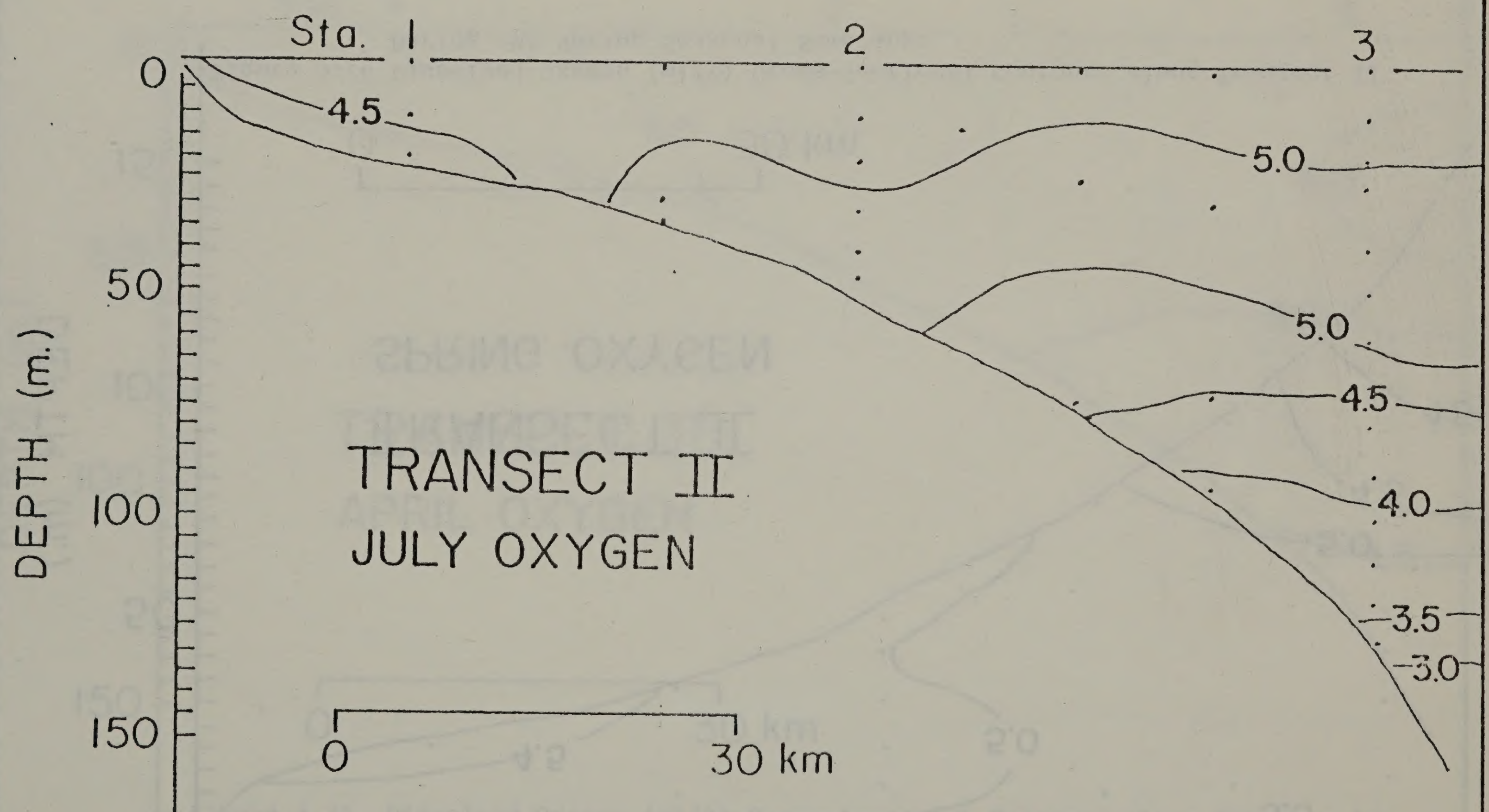

Figure 3.27 Dissolved Oxygen (ml/ℓ) Cross-Sectional Contours Along Transect II During the July Monthly Sampling.

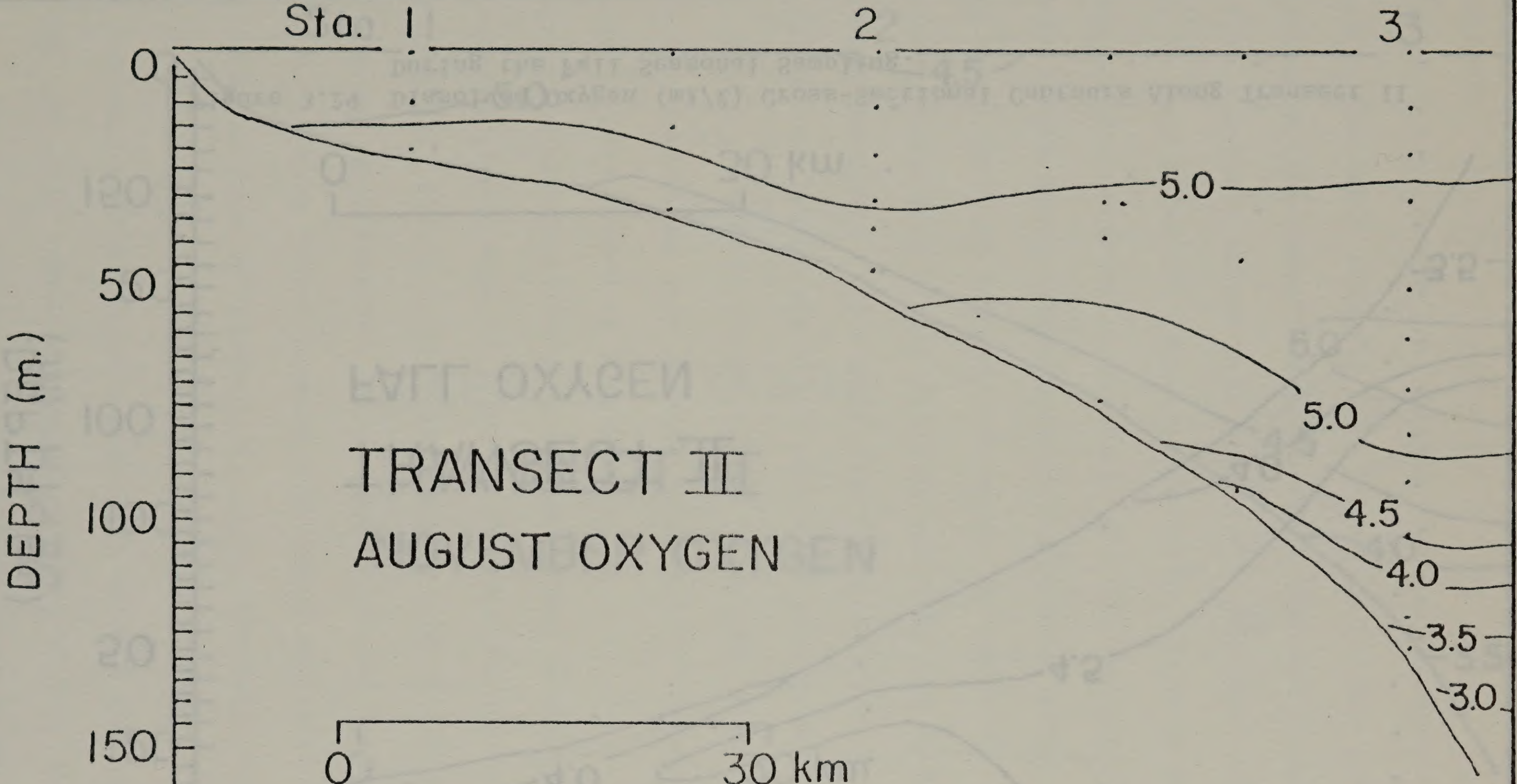

Figure 3.28 Dissolved Oxygen (ml/ℓ) Cross-Sectional Contours Along Transect II During the August Monthly Sampling.

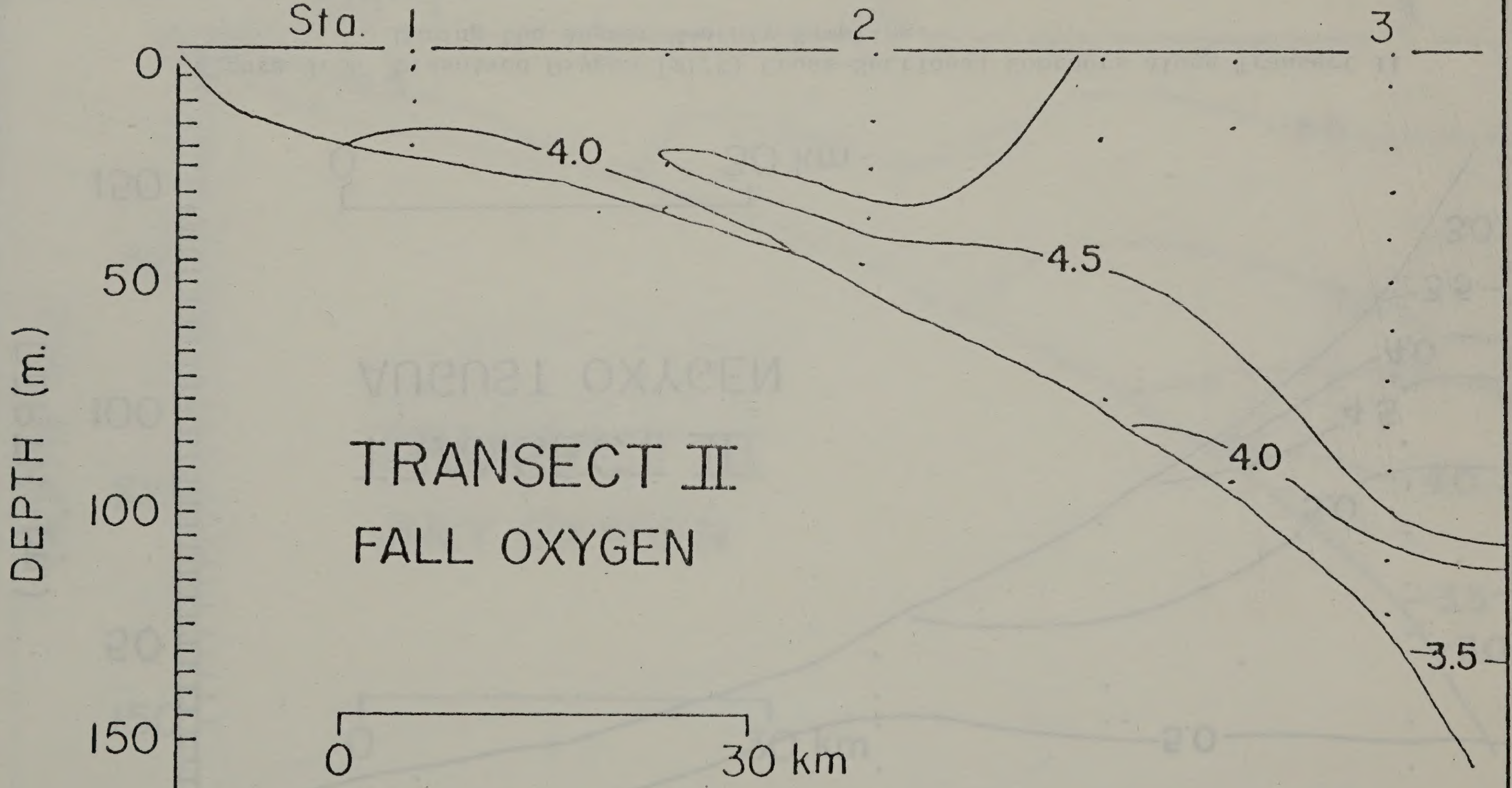

Figure 3.29 Dissolved Oxygen (ml/ℓ) Cross-Sectional Contours Along Transect II During the Fall Seasonal Sampling.

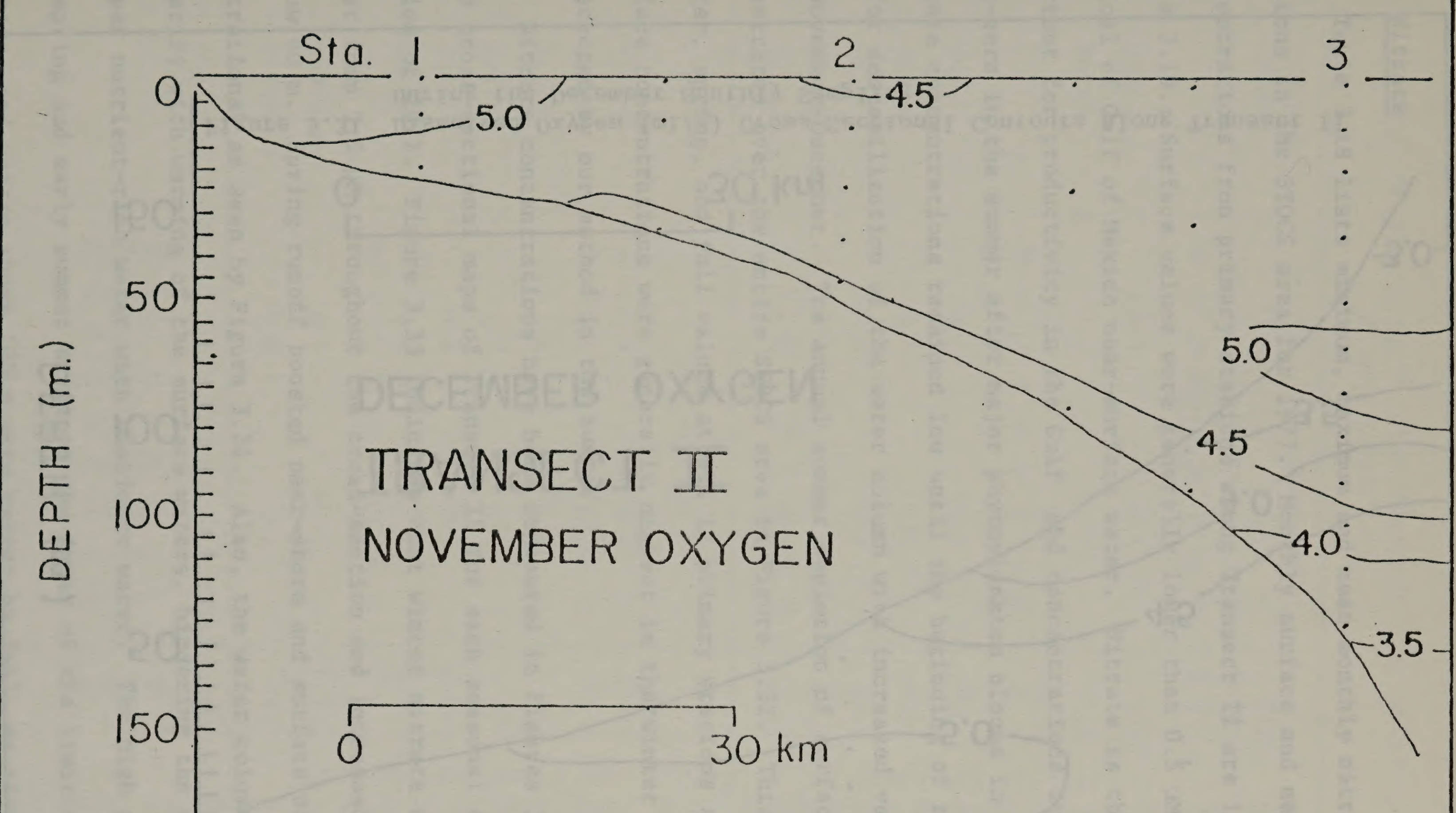

Figure 3.30 Dissolved Oxygen (ml/ℓ) Cross-Sectional Contours Along Transect II During the November Monthly Sampling.

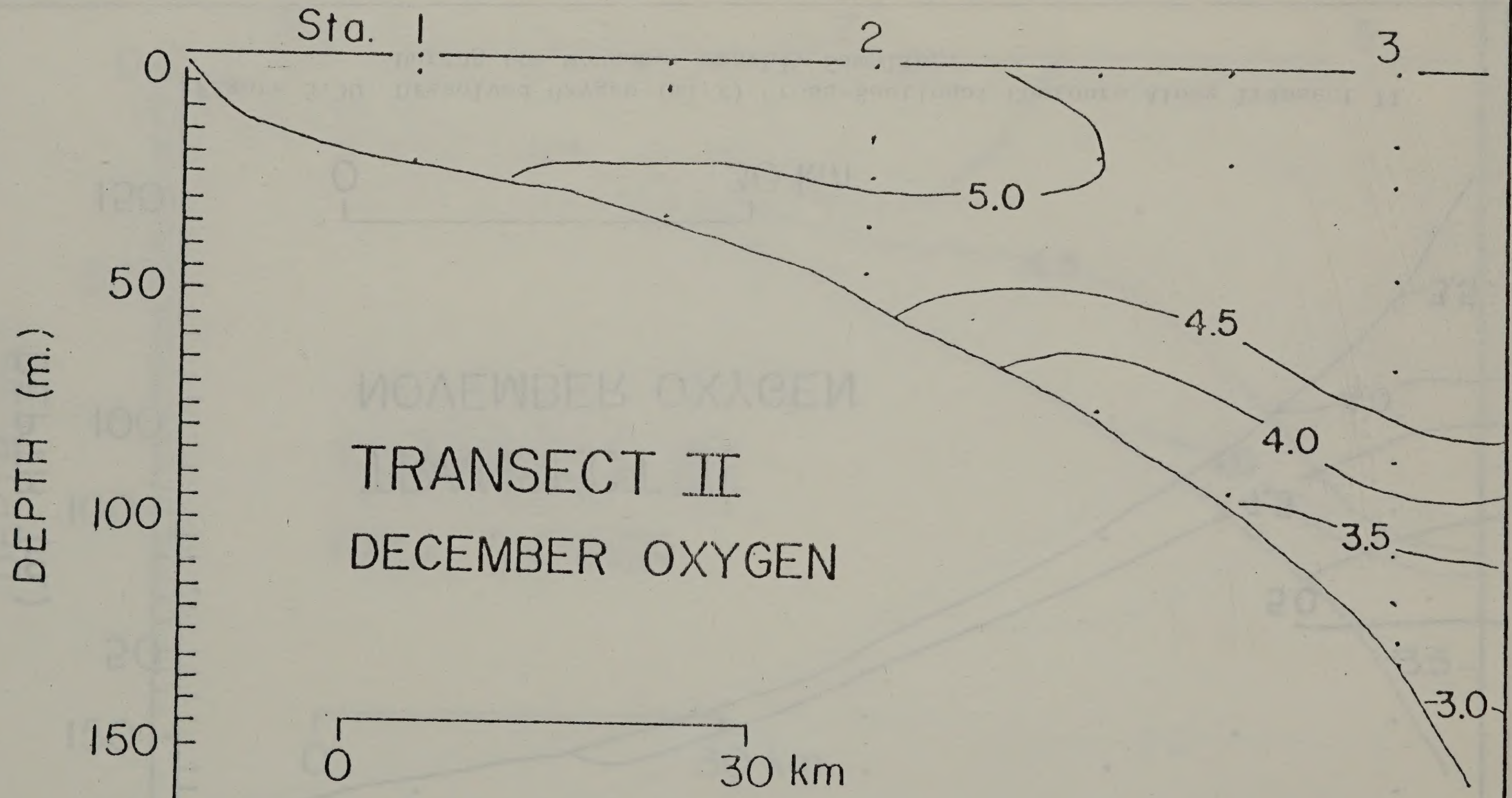

Figure 3.31 Dissolved Oxygen (ml/ℓ) Cross-Sectional Contours Along Transect II During the December Monthly Sampling.

cate are discussed separately.

Nitrate

Table 3.18 lists minimum, maximum and mean monthly nitrate concentrations in the STOCS area for 1977. Monthly surface and near-bottom concentrations from primary stations along Transect II are listed in Table 3.19. Surface values were generally lower than 0.5 µM, being typical of Gulf of Mexico near-surface water. Nitrate is the limiting nutrient for productivity in the Gulf and concentrations dropped to near-zero in the summer after major phytoplankton blooms in the spring. Nitrate concentrations remained low until the beginning of regeneration and/or destabilization of the water column with increased vertical mixing in November-December. The annual summer depletion of surface nitrate is illustrated over the entire STOCS area in Figure 3.32. This figure of winter, spring, and fall values at all 12 primary stations showed that surface concentrations were generally highest in the winter and almost undetected by our method in the summer.

Nitrate concentrations have been contoured in Figures 3.33 to 3.35 with cross-sectional maps of Transect II for each seasonal sampling period of 1977. Figure 3.33 indicates that winter nitrate was generally lower than 0.5 µM throughout the cross-section and increased moderately below 60 m. Spring runoff boosted near-shore and surface nitrate concentrations, as seen by Figure 3.34. Also, the water column began to stratify with warming of the surface waters, hindering the mixing of deeper nutrient-rich water with shallower water. The high productivity in spring and early summer stripped the water of the limiting nutrient, nitrate, and waters above 100 m were barren by fall, as seen by Figure 3.35. By this time the water column was highly stratified, inhibiting

TABLE 3.18

SUMMARY OF NUMBER OF NITRATE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (μM) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1977).

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	70	1.1	<0.1	7.7	0.8
March	9	0.5	<0.1	3.6	<0.1
April	15	0.8	<0.1	3.5	0.2
Spring	70	0.97	<0.1	4.8	0.3
July	19	1.5	<0.1	13.8	0.2
August	22	1.1	<0.1	9.3	0.1
Fall	93	0.5	<0.1	10.2	0.1
November	20	0.9	<0.1	11.2	0.1
December	18	2.7	0.2	11.8	0.5

TABLE 3.19

SURFACE AND NEAR-BOTTOM NITRATE CONCENTRATIONS (μM) AT STOCS STATIONS ALONG TRANSECT II.

SAMPLING MONTH	STATIONS 1/II	 2/II	 3/II
	Surface		
Winter	<0.1	0.3	0.3
March	0.1	<0.1	<0.1
April	0.5	0.1	0.1
Spring	0.2	0.2	0.2
July	0.4	0.2	<0.1
August	<0.1	<0.1	<0.1
Fall	0.1	<0.1	<0.1
November	0.1	<0.1	<0.1
December	0.5	0.8	0.3
	Near-Bottom		
Winter	0.4	0.1	7.7
March	0.2	0.2	3.6
April	0.6	0.3	0.2
Spring	1.3	0.4	2.8
July	0.4	<0.1	13.8
August	<0.1	<0.1	9.3
Fall	0.9	<0.1	5.8
November	0.9	<0.1	11.2
December	0.6	1.1	11.8

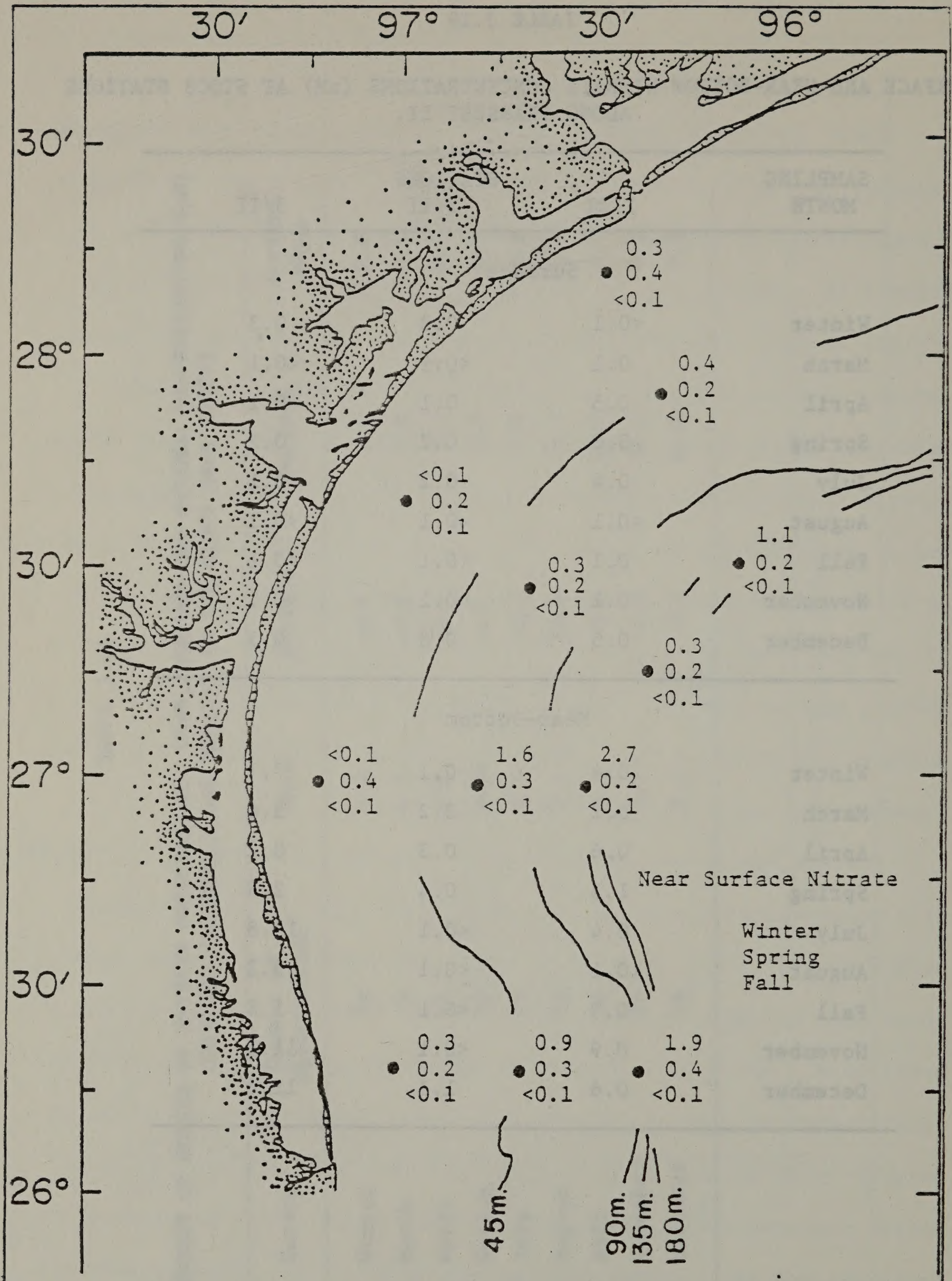

Figure 3.32 Near-Surface Nitrate Concentrations (μM) in the STOCS Area During the Seasonal Cruises in 1977.

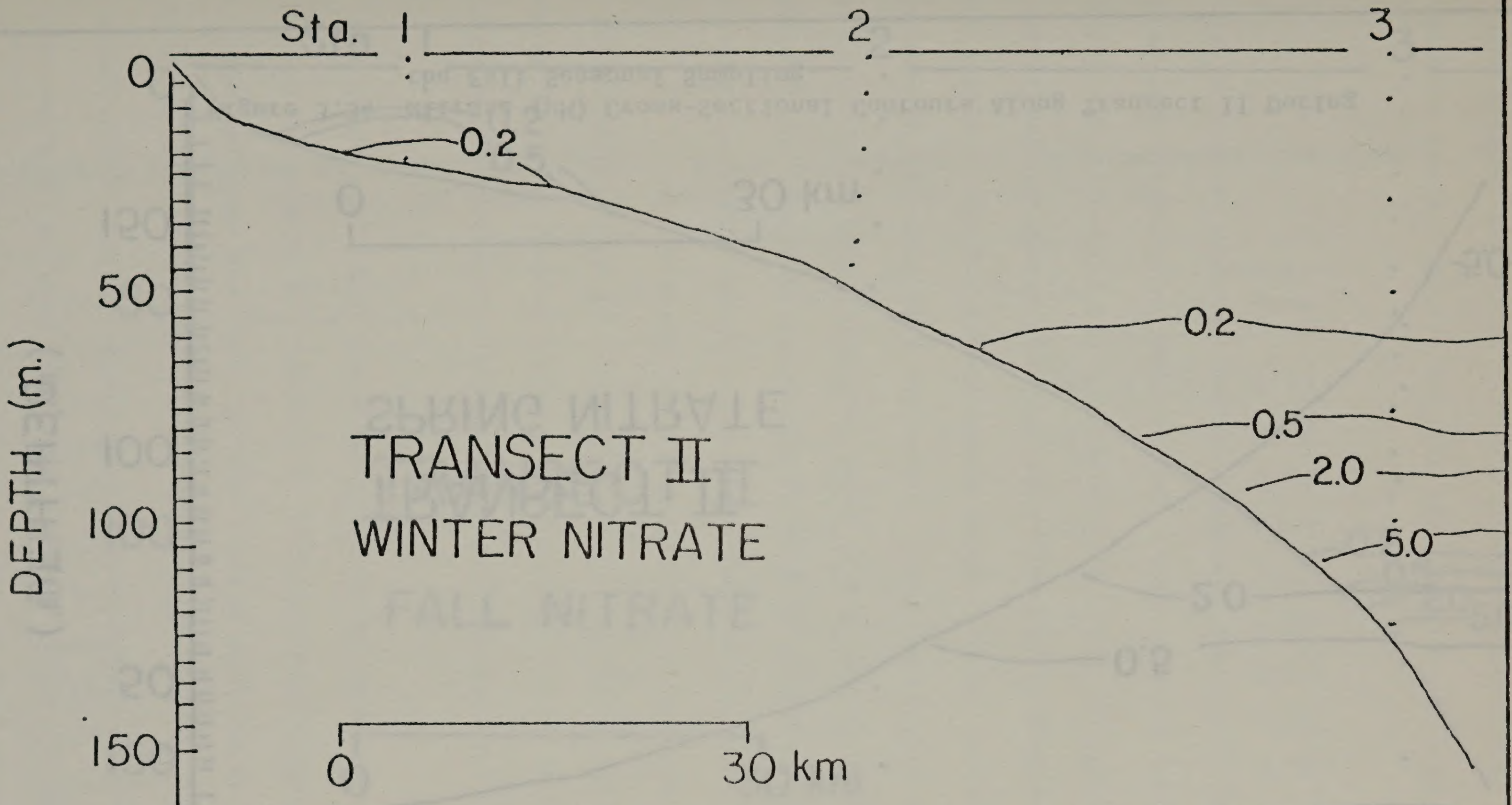

Figure 3.33 Nitrate (μM) Cross-Sectional Contours Along Transect II During the Winter Seasonal Sampling.

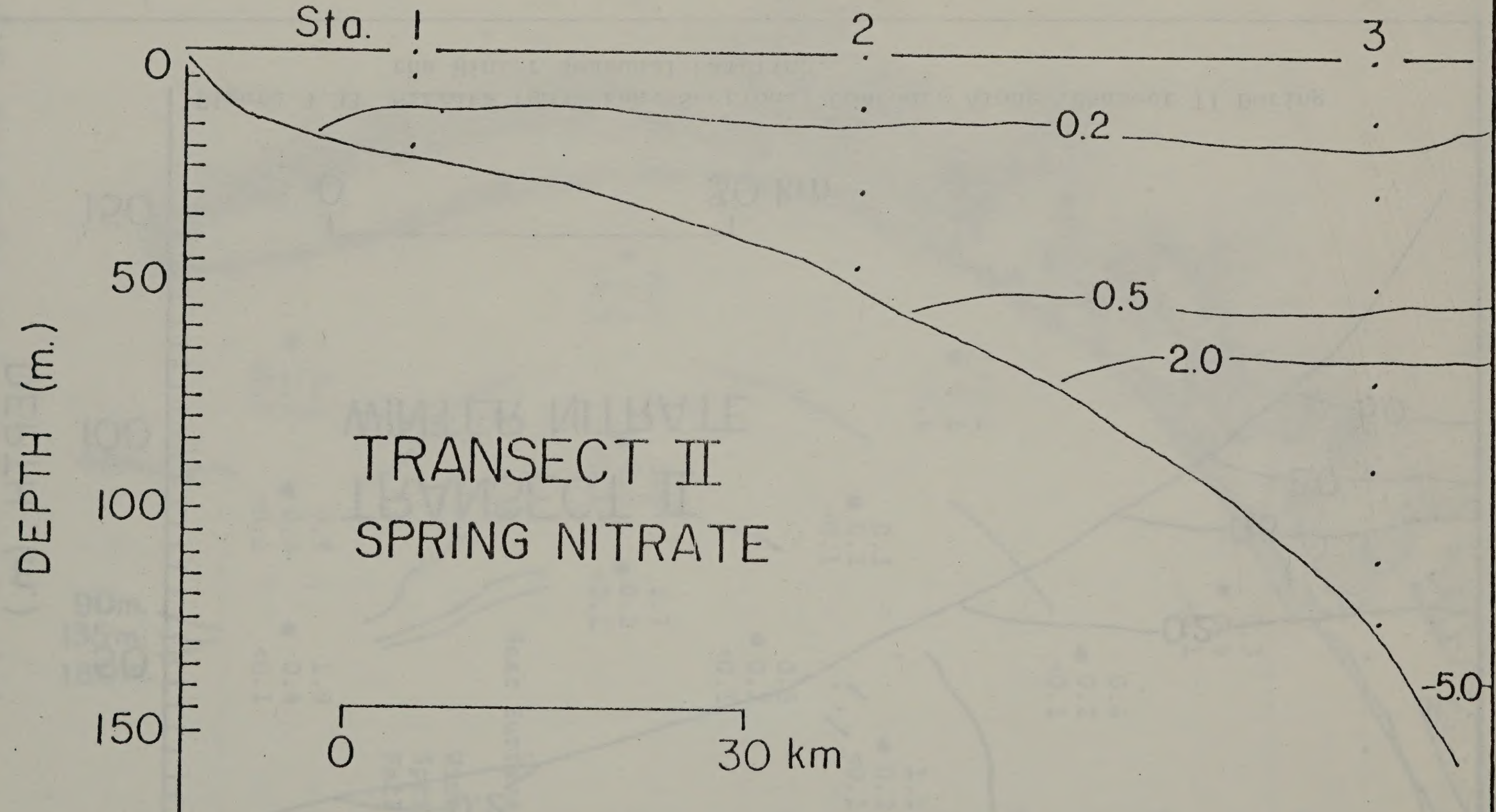

Figure 3.34 Nitrate (μM) Cross-Sectional Contours Along Transect II During the Fall Seasonal Sampling.

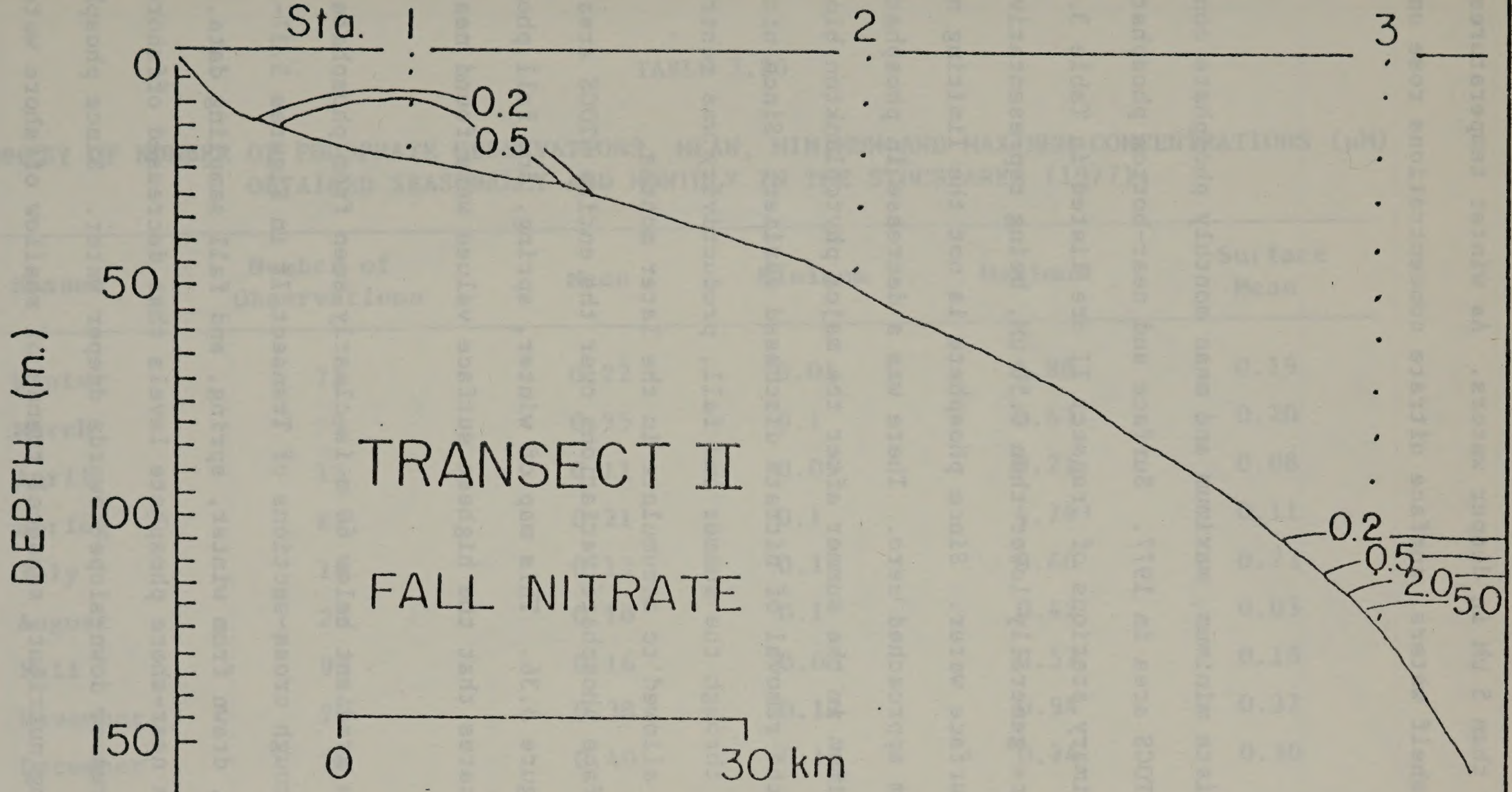

Figure 3.35 Nitrate (μM) Cross-Sectional Contours Along Transect II During the Fall Seasonal Sampling.

mixing of deep and shallow water, and nitrate concentrations rose rapidly to values greater than 5 µM in deeper waters. As winter temperatures cooled and destratified shelf waters, surface nitrate concentrations rose once again.

Phosphate

Table 3.20 lists minimum, maximum and mean monthly phosphate concentrations in the STOCS area in 1977. Surface and near-bottom phosphate levels for the primary stations of Transect II are listed in Table 3.21. Concentrations were generally lower than 0.50 µM, being representative of Western Gulf surface water. Since phosphate is not the limiting nutrient, values seldom approached zero. There was a decrease in phosphate, however, to a minimum in the summer after the major phytoplankton blooms, corresponding to the removal of nitrate discussed earlier. Since nitrate remained depleted through the summer and fall, productivity was restricted and phosphate was allowed to accumulate in the later months.

Seasonal surface phosphate variations over the entire STOCS area are illustrated by Figure 3.36. This map of winter, spring, and fall phosphate levels indicates that the highest surface values were found near-shore.

The phosphate gradient below 60 m is clearly seen from phosphate level contours through cross-sections of Transect II in Figures 3.37-3.39. The three figures, drawn from winter, spring, and fall sampling data, all exhibited the high near-shore phosphate levels that decreased offshore laterally and increased down-slope towards deeper water. Since phosphate is not the limiting nutrient, seasonal trends of shallow offshore water were not as obvious as with nitrate.

TABLE 3.20

SUMMARY OF NUMBER OF PHOSPHATE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (μM) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1977).

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	71	0.22	0.01	1.88	0.19
March	9	0.25	0.1	0.63	0.20
April	15	0.11	0.05	0.21	0.08
Spring	62	0.21	0.1	4.74	0.11
July	18	0.12	0.1	0.60	0.23
August	20	0.10	0.1	0.42	0.03
Fall	93	0.16	0.03	0.57	0.18
November	20	0.38	0.12	0.94	0.32
December	18	0.40	0.17	0.74	0.30

TABLE 3.21

SURFACE AND NEAR-BOTTOM PHOSPHATE CONCENTRATIONS (μM) AT STOCS STATIONS ALONG TRANSECT II.

SAMPLING MONTH	STATIONS 1/II	2/II	3/II
	Surface		
Winter	0.56	0.07	0.05
March	0.32	0.24	0.06
April	0.15	0.05	0.05
Spring	–	0.05	–
July	–	0.43	0.02
August	0.03	0.06	<0.01
Fall	0.42	0.12	0.06
November	0.46	0.27	0.24
December	0.40	0.29	0.20
	Near-Bottom		
Winter	1.34	0.24	0.41
March	0.63	0.20	0.38
April	0.17	0.06	0.10
Spring	0.25	0.17	0.17
July	0.23	0.04	0.60
August	0.16	0.06	–
Fall	0.35	0.16	0.34
November	0.46	0.28	0.64
December	0.73	0.24	0.74

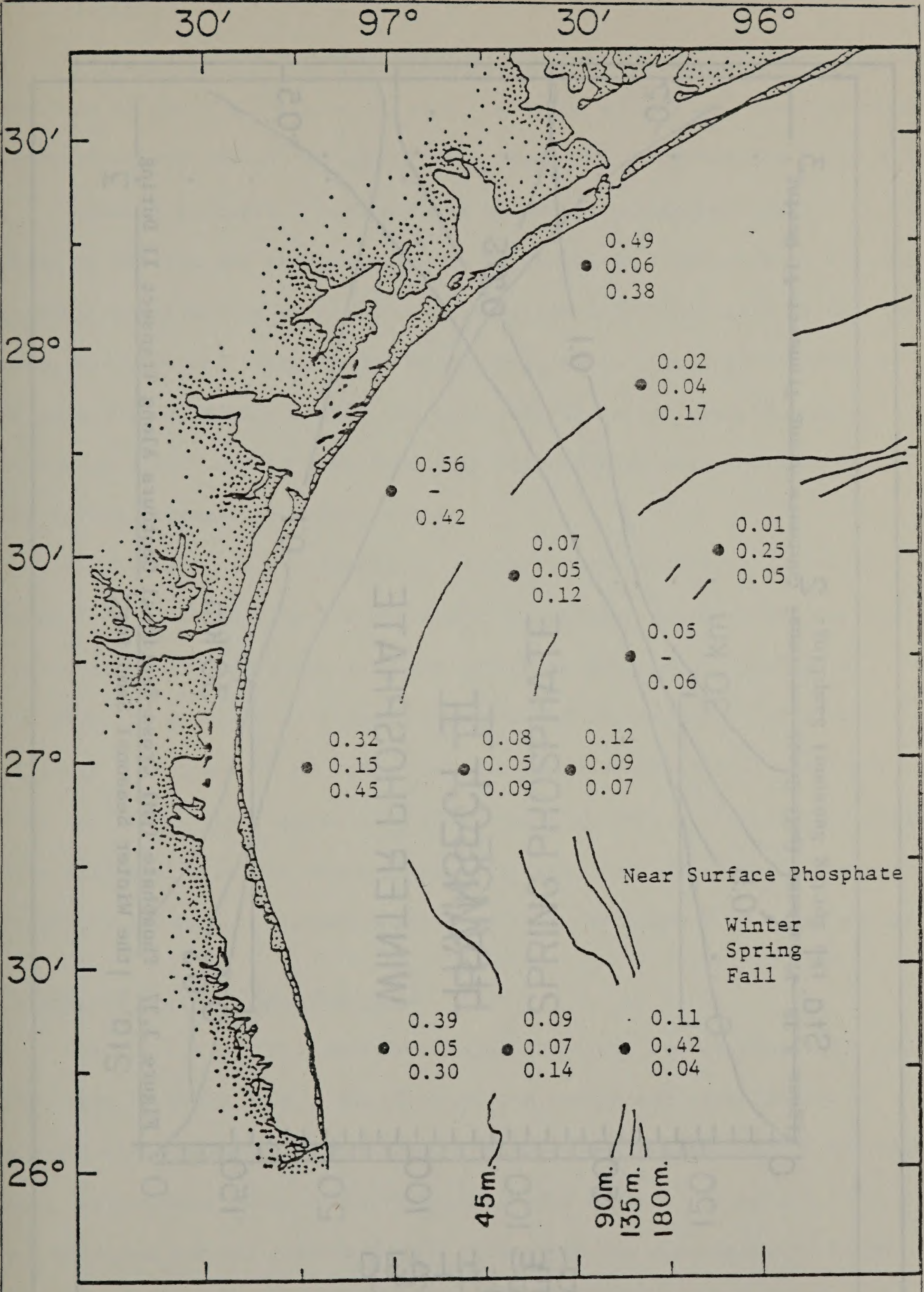

Figure 3.36 Near-Surface Phosphate Concentrations (μM) in the STOCS Area During the Seasonal Cruises in 1977.

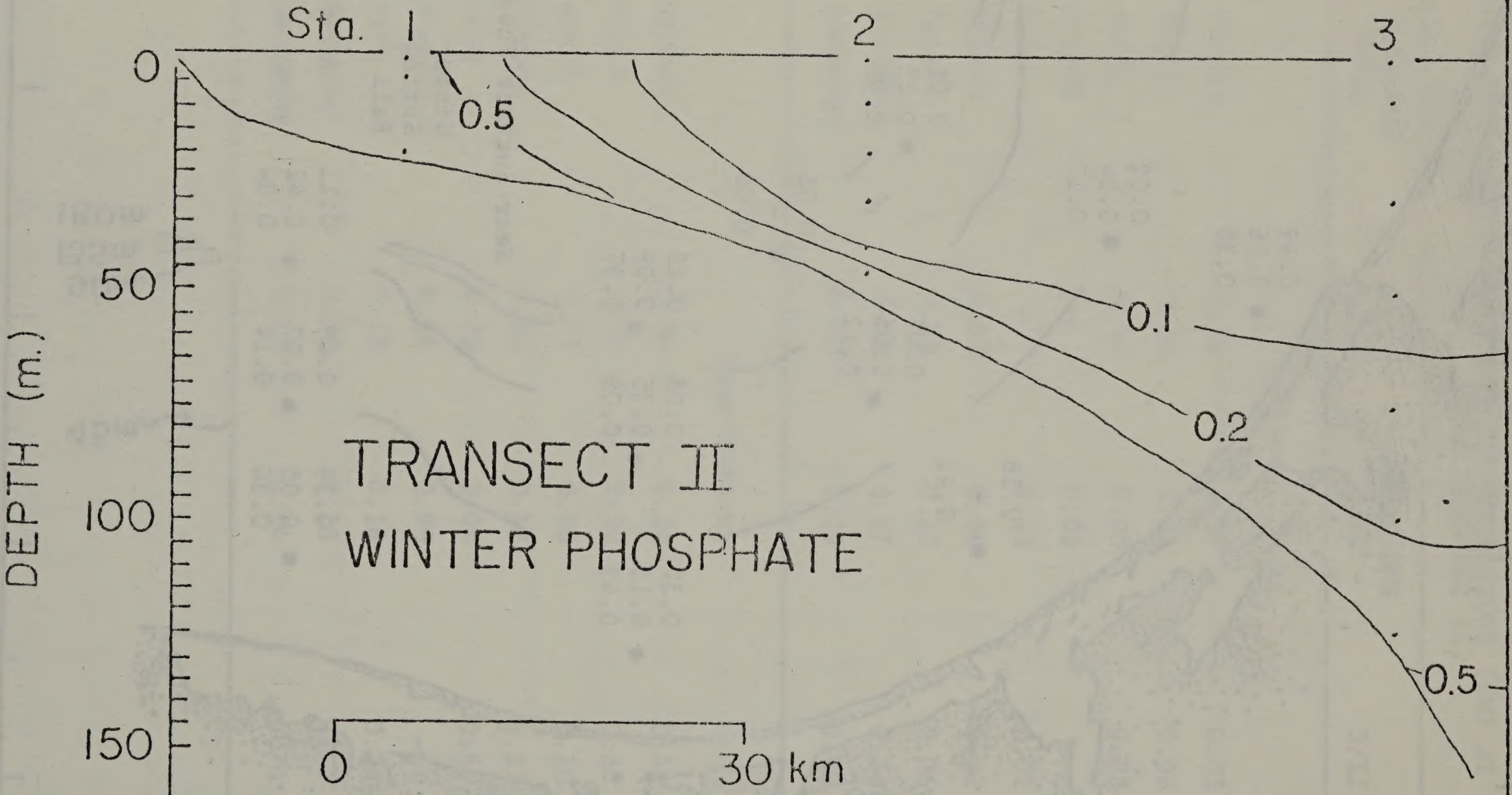

Figure 3.37 Phosphate (μM) Cross-Sectional Contours Along Transect II During the Winter Seasonal Sampling.

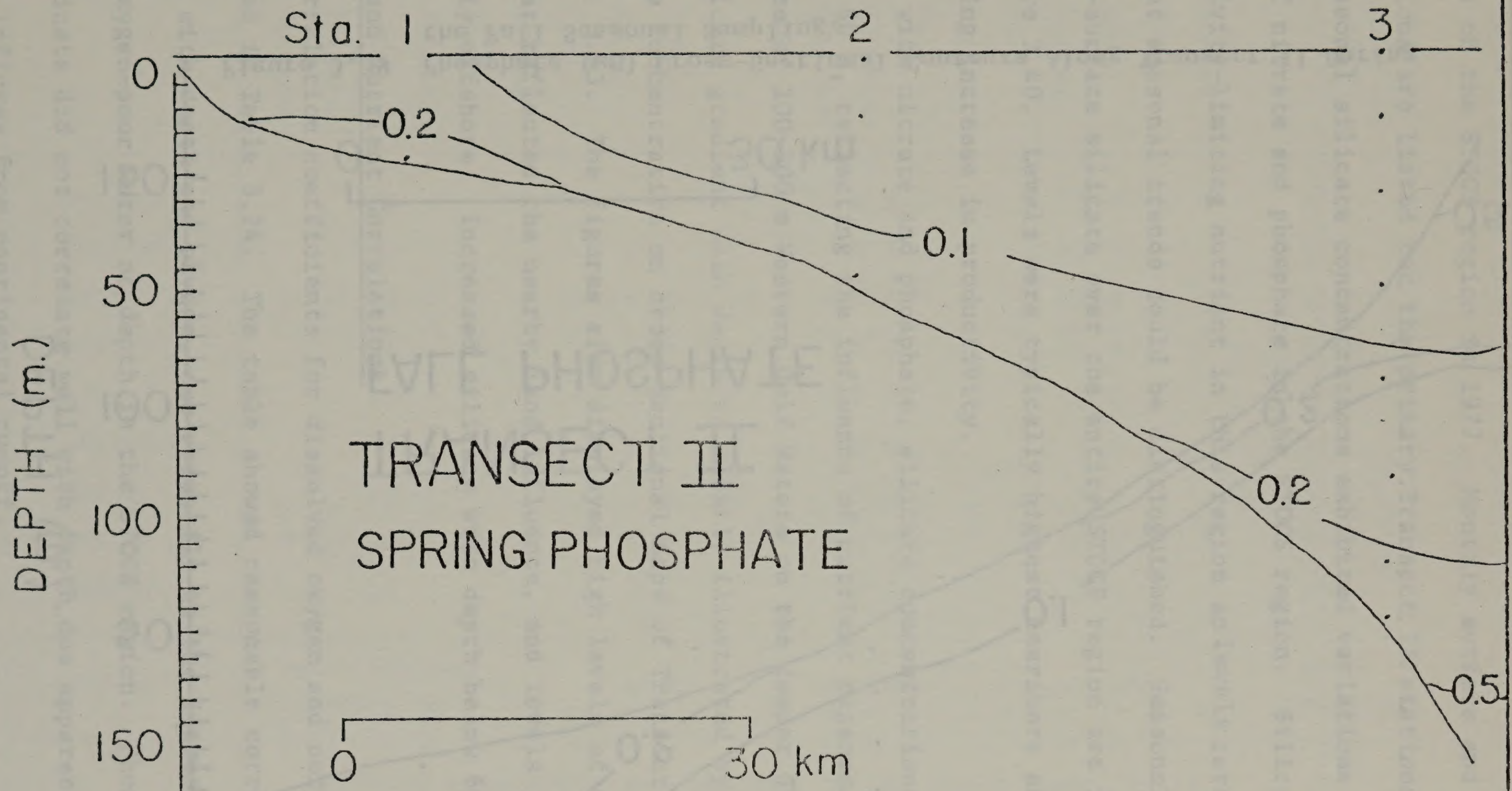

Figure 3.38 Phosphate (μM) Cross-Sectional Contours Along Transect II During the Spring Seasonal Sampling.

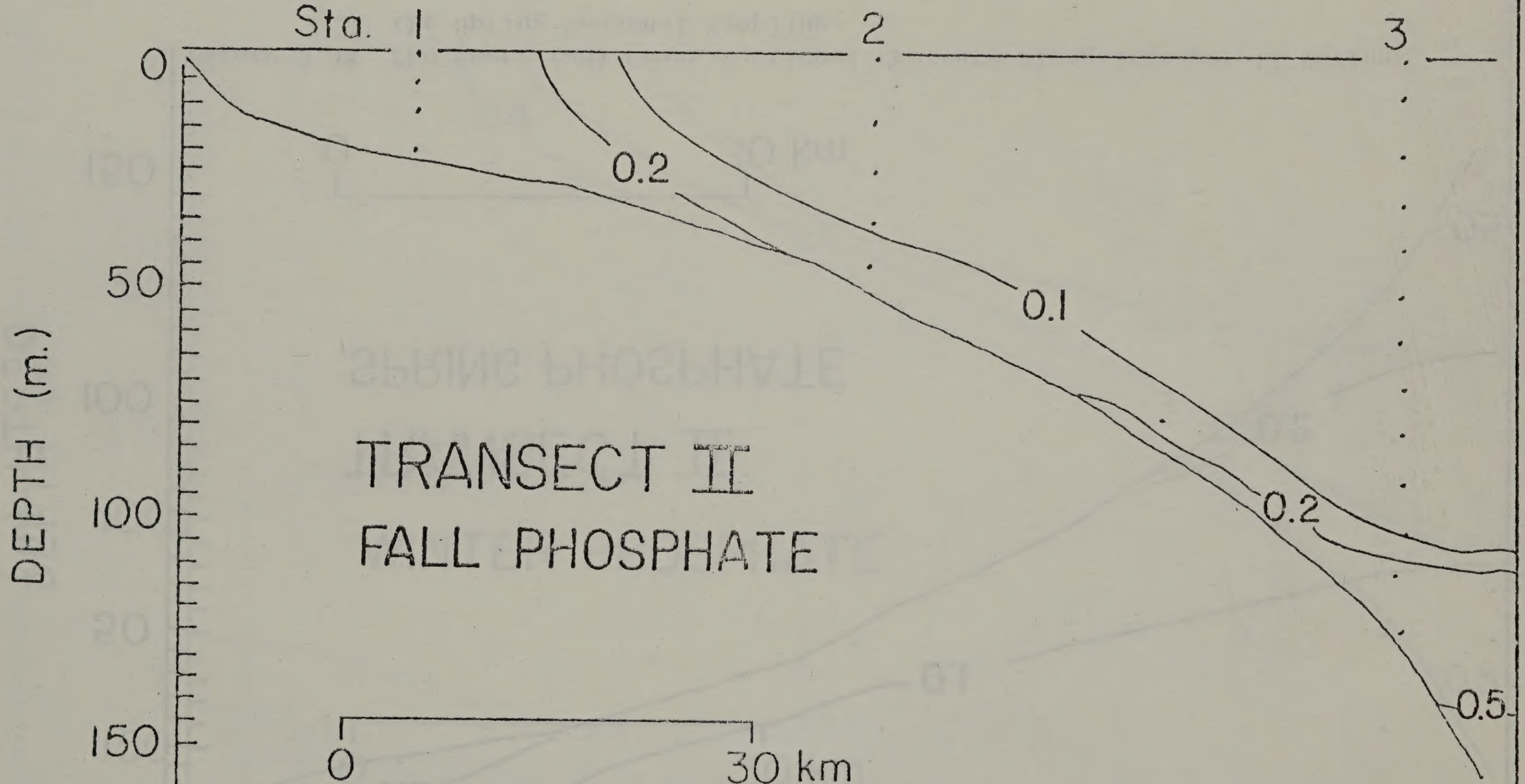

Figure 3.39 Phosphate (μM) Cross-Sectional Contours Along Transect II During the Fall Seasonal Sampling.

Silicate

Table 3.22 lists minimum, maximum, and mean monthly silicate concentrations of the STOCS region in 1977. Monthly surface and near-bottom concentrations are listed for the primary Transect II stations in Table 3.23.

Seasonal silicate concentrations exhibited variations similar to those of nitrate and phosphate in the STOCS region. Silicate is not the productivity-limiting nutrient in this region so levels rarely approached zero, but seasonal trends could be distinguished. Seasonal variations of near-surface silicate over the entire STOCS region are illustrated in Figure 3.40. Levels were typically highest nearshore and lowest during the spring increase in productivity.

As with nitrate and phosphate, silicate concentrations increased below ~ 60 m, reflecting the influence of nutrient regeneration and the influence of 200-300 m Western Gulf Waters on the deeper STOCS water. The silicate gradient with depth is clearly illustrated by contours of silicate concentration on cross-sectional maps of Transect II in Figures 3.41 to 3.43. The figures also displayed high levels of nearshore silicate that reflected the nearby land influence, and levels that decreased laterally offshore. Increased silicate with depth below 60 m was evident.

Oxygen and Nutrient Correlations

Correlation coefficients for dissolved oxygen and nutrient data are tabulated in Table 3.24. The table showed reasonable correlations of nitrate with depth and oxygen, due primarily to the intrusion of nutrient-rich, oxygen-poor water at depth in the STOCS region. Phosphate and silicate did not correlate well with depth, due apparently to large surface influxes from continental runoff.

TABLE 3.22

SUMMARY OF NUMBER OF SILICATE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (µM) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1977).

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	71	1.7	0.2	5.2	1.7
March	9	1.9	<0.1	4.7	1.6
April	15	2.8	0.8	6.7	3.1
Spring	70	2.0	0.4	7.5	1.2
July	19	2.3	1.3	5.8	2.2
August	21	1.9	0.8	6.3	1.6
Fall	93	4.2	1.4	13.9	5.5
November	20	4.6	2.3	12.7	5.6
December	18	4.3	2.3	7.5	5.0

TABLE 3.23

SURFACE AND NEAR-BOTTOM SILICATE CONCENTRATIONS (µM) AT STOCS STATIONS ALONG TRANSECT II.

SAMPLING MONTH	STATIONS 1/II	2/II	3/II
		Surface	
Winter	2.3	1.4	0.5
March	3.7	1.1	0.1
April	6.2	1.3	1.7
Spring	2.5	0.8	0.8
July	3.3	1.7	1.7
August	1.3	1.7	1.7
Fall	11.9	3.4	2.4
November	9.2	4.7	2.8
December	6.8	4.9	3.4
		Near-Bottom	
Winter	2.2	1.6	4.3
March	4.7	1.2	2.2
April	6.2	1.7	0.8
Spring	6.7	3.3	3.3
July	3.3	2.1	3.8
August	5.0	1.7	6.3
Fall	6.7	4.2	3.5
November	12.7	4.8	5.3
December	7.5	3.5	5.0

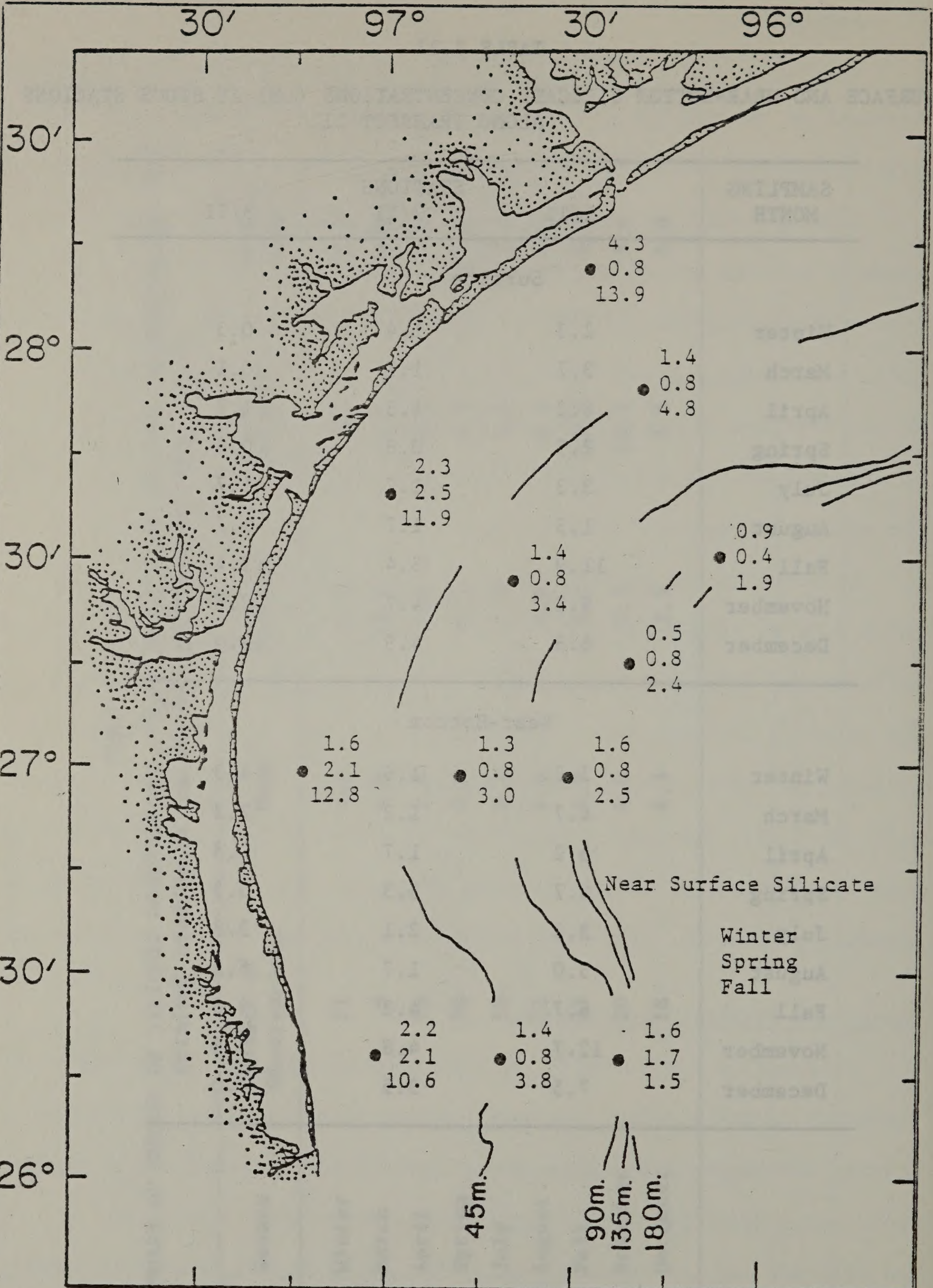

Figure 3.40 Near-Surface Silicate Concentrations (μM) in the STOCS Area During the Seasonal Cruises in 1977.

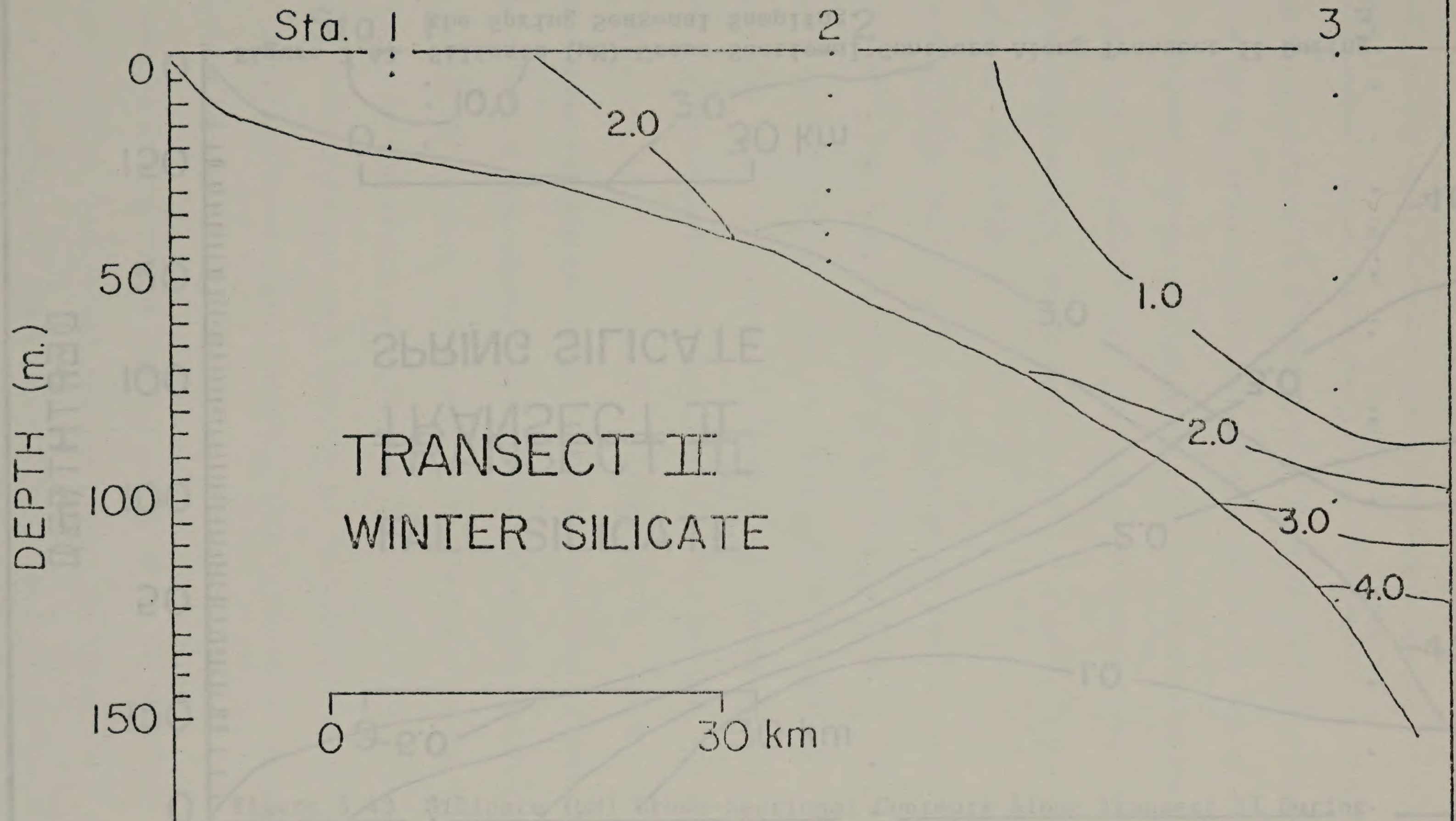

Figure 3.41 Silicate (μM) Cross-Sectional Contours Along Transect II During the Winter Seasonal Sampling.

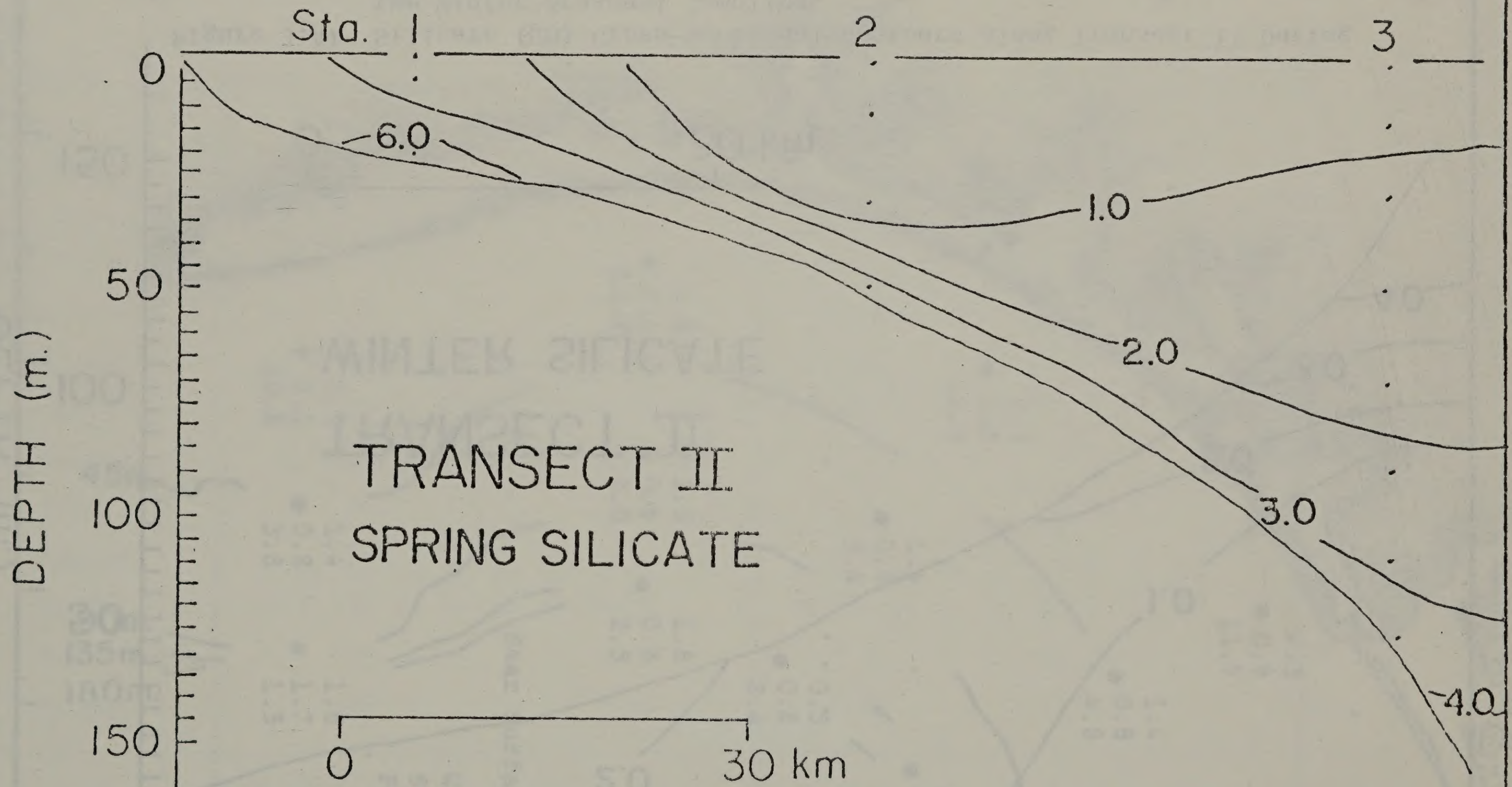

Figure 3.42 Silicate (μM) Cross-Sectional Contours Along Transect II During the Spring Seasonal Sampling.

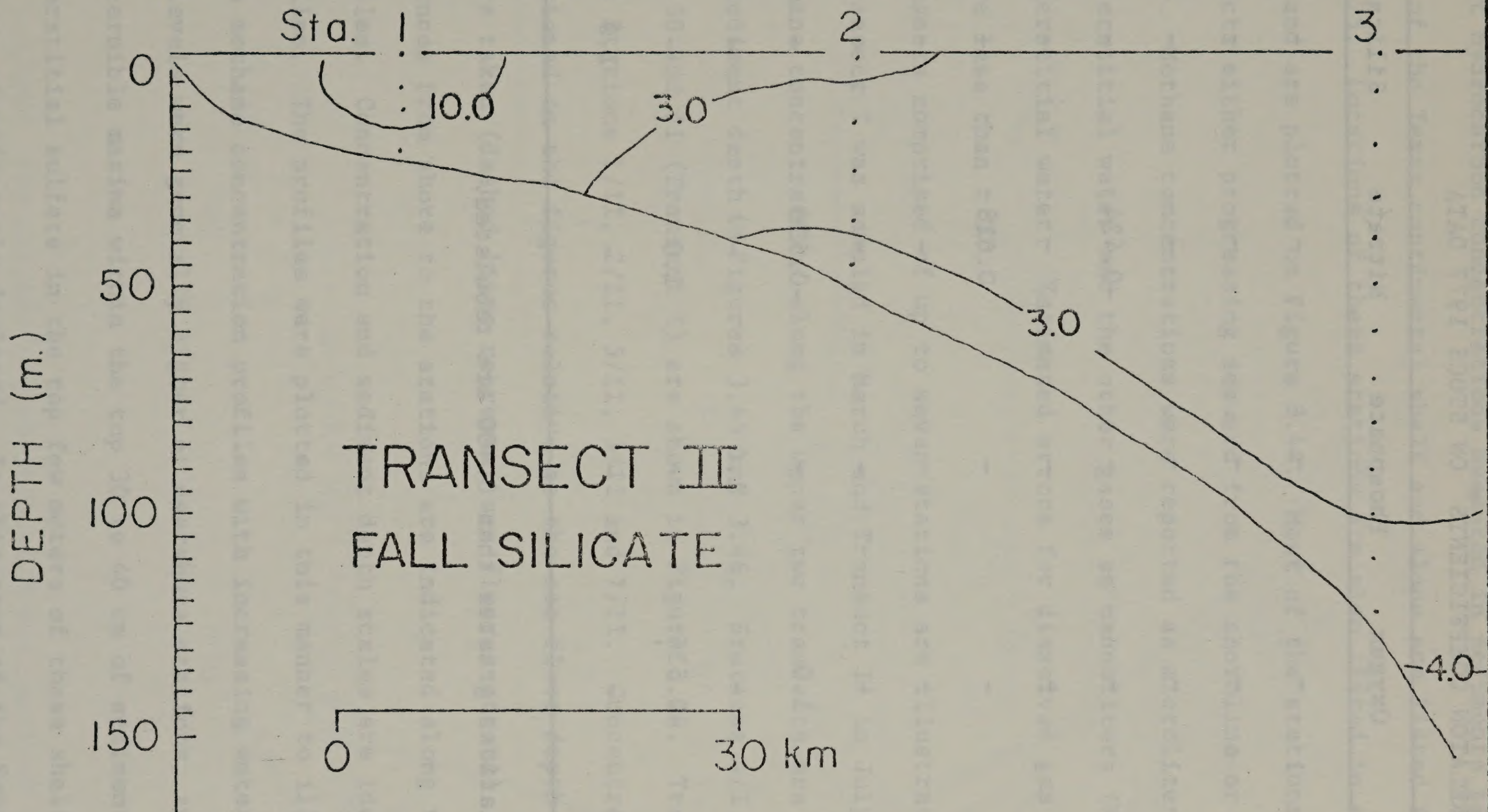

Figure 3.43 Silicate (μM) Cross-Sectional Contours Along Transect II During the Fall Seasonal Sampling.

Table 3.24

CORRELATON COEFFICIENTS[†] ON STOCS 1977 DATA

Variable	Oxygen	Phosphate	Nitrate	Silicate
Methane	-	-	-	-
Ethene	-	-	-	-
Ethane	-	-	-	-
Propene	-	-	-0.424	-
Propane	-	-	-	-
Depth	-	-	0.618	-
Temperature	-	-	-	-
Salinity	-	-	-	-
Oxygen	1.0	-	-0.536	-
Phosphate	-	1.0	-	-
Nitrate	-0.536	-	1.0	-
Silicate	-	-	-	1.0

[†] Only correlations greater than 0.400 are tabulated.

Sediment Low-Molecular Weight Hydrocarbons

Light hydrocarbon concentrations measured in sediments taken at 53 stations of the Texas continental shelf and slope are listed in Appendix B, Table 25. Locations of these stations are also listed in Appendix B, Table 25 and are plotted on Figure 3.44. Most of the stations were located on transects either progressing seaward from the shoreline or tangent to the coast. Methane concentrations were reported as microliters (NTP) per liter interstitial water and the other gases as nannoliters (NTP) per liter interstitial water. Estimated errors for dissolved gas determinations were less than ± 3%.

Transects comprised of up to seven stations are illustrated in Figure 3.44. Transect I was sampled in March and Transect II in July. Interstitial methane concentrations along the upper two transects are plotted against sediment depth in Figures 3.45 and 3.46. Stations 4/I, 2/I, 5/I, 3/I, 49, 50 and 51 (Transect I) are shown in Figure 3.45. Transect II contained Stations 1/II, 2/II, 5/II, 3/II and 7/II. Concentration profiles are positioned on the figures relative to the sea floor depth where the cores were taken (dashed lines represent sea floor contours). Water depths and distances from shore to the stations are indicated along the axes of the profiles. Concentration and sediment depth scales are identical in all profiles. The profiles were plotted in this manner to illustrate the change in methane concentration profiles with increasing water depth. Methane levels were generally higher at nearshore stations, and showed very discernible maxima within the top 30 to 40 cm of sediment.

Interstitial sulfate in the top few meters of these shelf sediments had not been significantly depleted. In this area of the South Texas shelf sulfate has been found down to several meters below the sediment surface, so methane should not have been produced so extensively in the

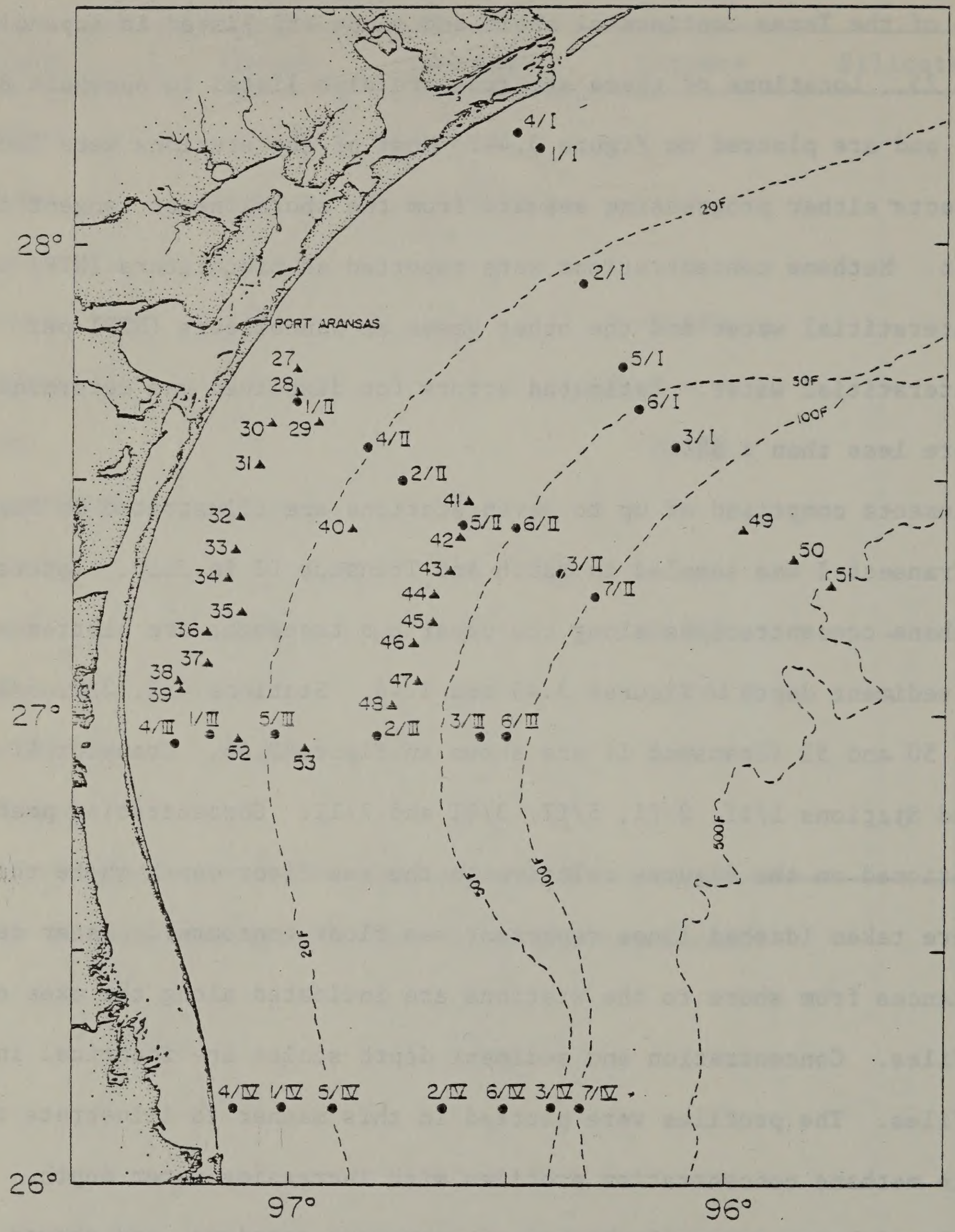

Figure 3.44 Sediment Low-Molecular-Weight Hydrocarbon Sampling Locations on the South Texas Shelf.

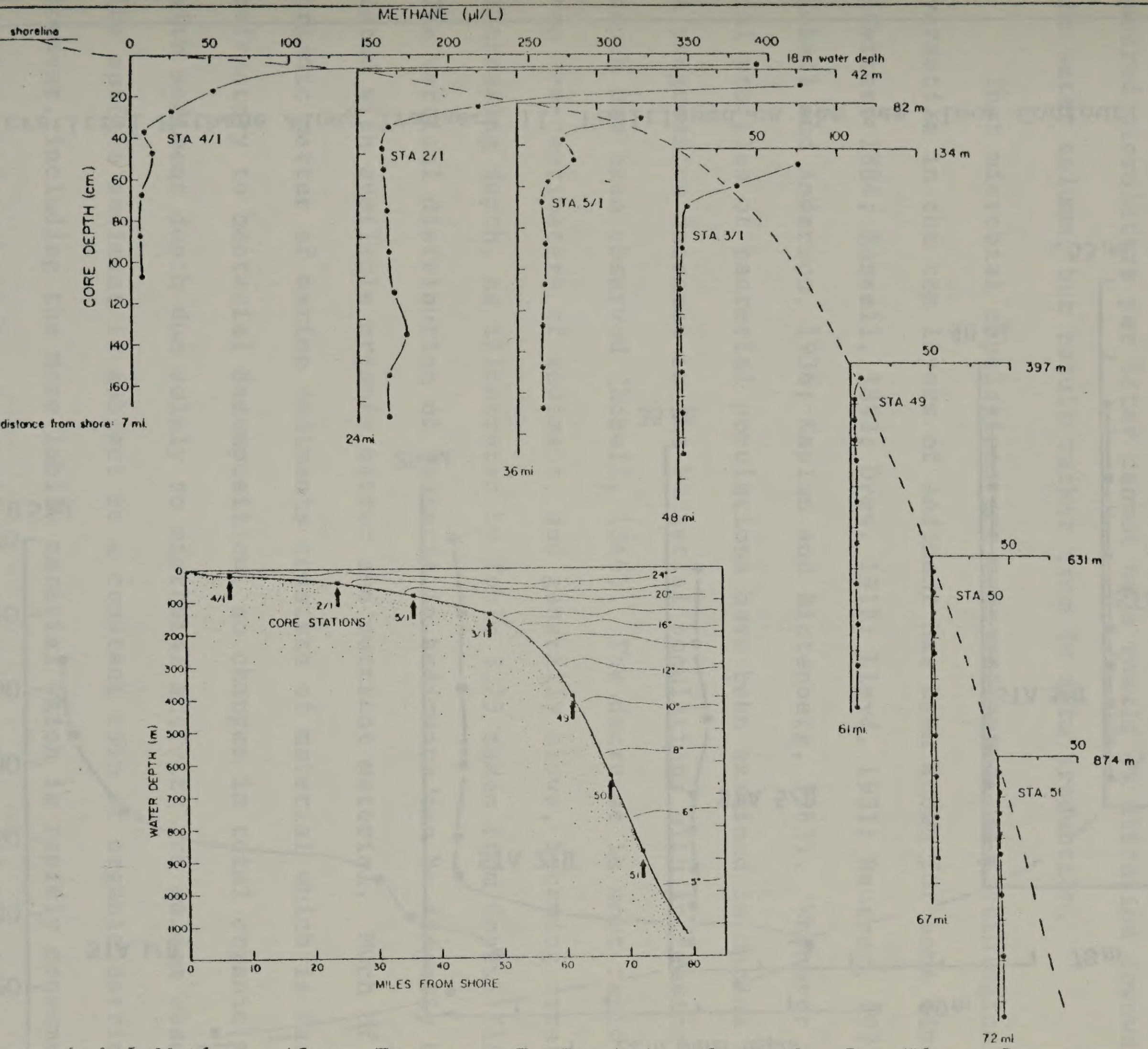

Figure 3.45 Interstitial Methane Along Transect I, Positioned on the Sea Floor Contour. Inset Shows the General Temperature Structure of the Area in Spring.

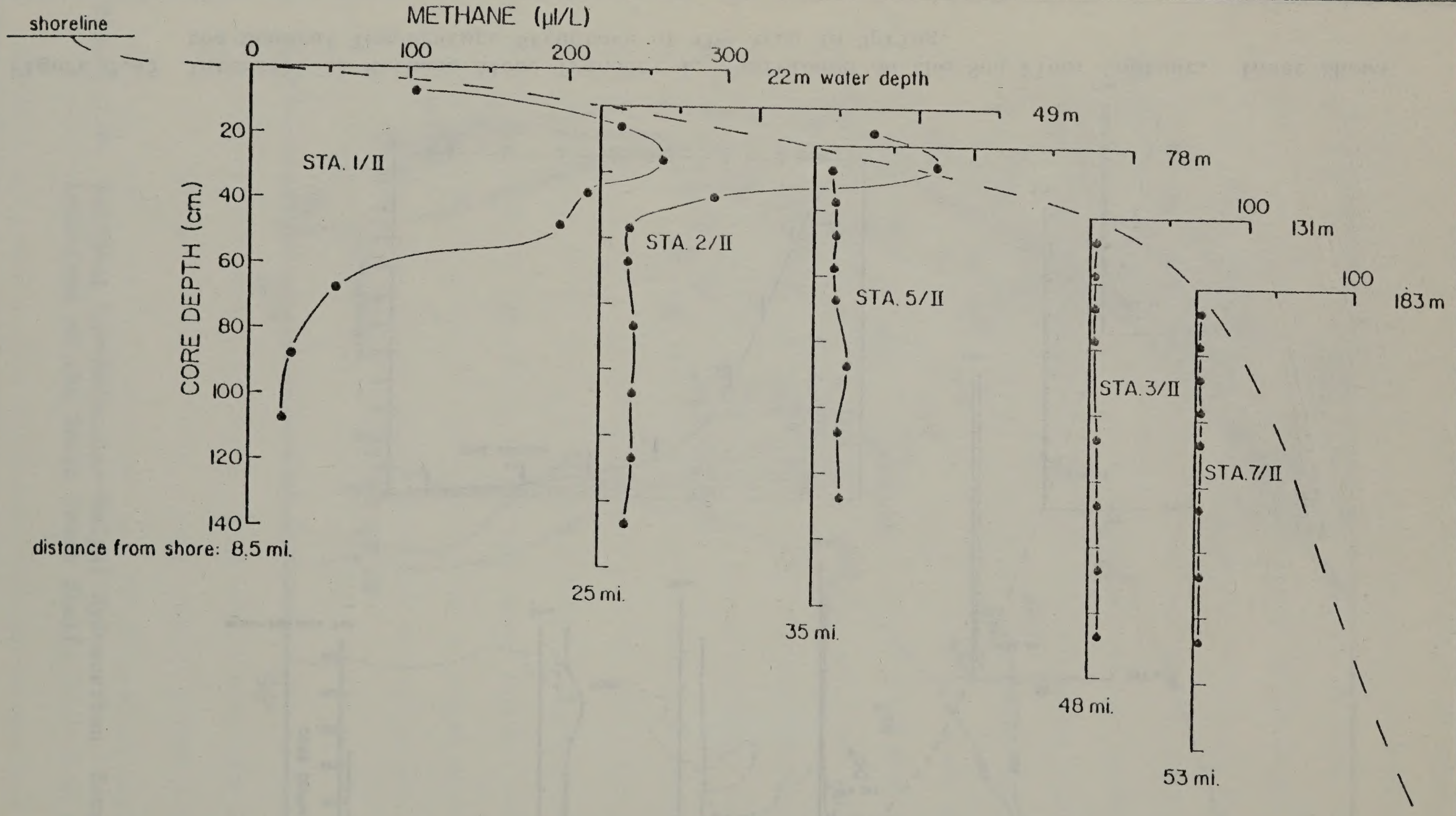

Figure 3.46 Interstitial Methane Along Transect II, Positioned on the Sea Floor Contour.

surface sulfate-rich sediments.

Methane maxima in near-bottom waters have been consistently observed in some areas, but few concentrations above 0.4 μℓ/ℓ have been recorded. Therefore, methane concentrations in surface sediments as high as several hundred microliters per liter cannot be a result of diffusion downward from the water column, but result rather from *in situ* production.

That microbial populations are most extensive and physiologically versatile in the top layers of sediment has been known for some time (Certes, 1884; Russell, 1892; Drew, 1912; Lloyd, 1931; Reuszer, 1933; Zobell and Anderson, 1936; Kaplan and Rittenberg, 1963). Whenever vertical profiles of bacterial populations have been examined in marine sediments, a progressive decrease in the bacterial populations with increasing sediment depth has been observed (Zobell, 1946). The decrease is most rapid in the top few centimeters of sediment, and generally slows, becoming erratic with increasing depth, as illustrated by Table 3.25 taken from Zobell (1942). The vertical distribution of bacteria in sediments can be directly correlated with available organic matter and nutrient material. Much of the organic matter of marine sediments consists of material which is fairly refractory to bacterial decomposition, so changes in total organic matter with sediment depth due solely to microbial activity are seldom observable. The surface sediment is subject to a constant rain of organic detritus, however, including the more labile material which is rapidly consumed by bacteria before burial. As available organic matter and nutrients disappear with depth in the sediment, bacterial populations decrease.

There were obvious similarities between vertical bacterial distributions and the vertical methane profiles of nearshore stations illustrated in Figures 3.45 and 3.46. The two figures suggested that the methanogenic

TABLE 3.25

BACTERIA PER GRAM SEDIMENT AT THREE CORE SITES OFF THE CALIFORNIA COAST (ZOBELL, 1972)

Core Depth (cm)	Bacteria per gram	Bacteria per gram	Bacteria per gram
0-2	840,000	38,000,000	7,500,000
3-5	102,000	940,000	250,000
10-12	63,000	88,000	160,000
23-25	19,000	36,000	23,000
36-38	1,500	2,400	8,700
48-50	2,200	400	2,100
74-76	370	180	600
99-101	190	330	200
150-152	210	250	300
201-203	140	130	100
252-254	140	290	150
Water depth	430m	950m	1090m

bacteria existed in large numbers near the sediment surface, and were possibly active inside small, sulfate-free microenvironments in the sediment after reduction of ambient sulfate had occurred. These micro-niches could have taken the form of fecal pellets, decaying organic matter, shell fragments, or flocculent clay particles. Methane produced in the microenvironments apparently diffused into the surrounding sediment, where it was bacterially oxidized, and diffused upward into the overlying bottom water where it was removed by advection.

The shelf stations taken along Transect I, II and III (Figures 3.45 and 3.46) were at similar water depths and distances from shore. Consequently if only these factors affected methane concentrations, corresponding profiles in the two figures should ideally have been identical. This was not the case since the profiles along Transect I showed that methane increased upward to the 5-10 cm interval, whereas the profiles of Transect II indicated a methane maximum at the 25-30 cm interval (concentrations in the 0.5 cm interval are unknown).

The lack of uniformity in the distribution of bacteria among the various sediment types observed on the Texas shelf might have explained these differences. Bacterial distributions were intimately associated with the physical consistency and organic content of the sedimentary deposits. In some areas, submarine topography had a greater influence on the median particle size and organic content of the sediments than the depth of water or distance from shore. Zobell (1946) stated that bacterial populations were more closely related to the character of the sediments than to their distance from land and, as a rule, sand contained fewer bacteria than sediments consisting of smaller particles. The greater abundance of bacteria found in finer sediments was attributed primarily to a higher organic content.

The absence of high surface methane concentrations along Transect II could also have been attributed to seasonal influences. Transect II was sampled in July, whereas Transect I was sampled in March. Nearshore water temperatures approaching 30°C in the summer months may have been above the temperature range for optimum growth of the methanogens, effectively inhibiting methane production. Other possible seasonal effects included changes in fluxes of organic detritus and nutrients to the surface sediments.

Figures 3.45 and 3.46 also illustrate the disappearance of the surface methane maximum in cores taken progressively further offshore. Figure 3.45 shows that the methane maximum decreased significantly at Station 3/I, was barely visible at Station 49, and disappeared at Stations 50 and 51. The decrease of the near-surface methane with increasing distance from shore could be explained by changes in microbial activity rather than changes in populations. Bacterial numbers generally decrease outward on the Texas continental shelf (J. Schwarz, personal communication), although millions of bacteria per gram of sediment are still found in sediments several thousands of meters deep. Therefore, the observed decrease of the methane maximum could not be explained on the basis of bacterial numbers alone.

In some areas there are actually more bacteria in sediments from deep water, where the temperatures are 3-7°C, than in those from shallow waters, where bottom temperatures are considerably higher (Zobell and Anderson, 1936). The optimum temperatures for the multiplication of marine bacteria probably range from 20-25°C, but while lower temperatures retard reproduction, survival of the bacteria is prolonged.

General water temperature contours at the study area are shown in the insert of Figure 3.45. These temperatures were representative of late spring but did not change significantly below 200 m throughout the year. Stations were marked on the insert and surface methane concentrations correlated well with the temperature contours. Temperatures below ~ 15°C and decreased labile organic inputs beyond the shelf break apparently inhibited microbial activity and methane production to an extent that methane diffused out of the sediment before it could accumulate as it did nearer shore. The increase in hydrostatic pressure may also have suppressed production of methane in these slope sediments. For example, Jannasch *et al.* (1971) found that rates of microbial activity were 10 to 100 times slower in the deep-sea than in controls at comparable temperatures.

Changes in production with temperature again implied that surface methane concentrations in Texas shelf sediments were seasonally influenced. Low temperatures nearshore in winter (~ 12°C) could have inhibited microbial activity and slowed methane production. Warming of the sediments in the spring, enhanced by increased detrital input from phytoplankton blooms and runoff, might have accelerated methane production in the microenvironments, causing methane oscillations at the tops of nearshore sediments. These oscillations, if real, could have provided an excellent means of evaluating the magnitude of the effective diffusion coefficient of methane in porous sediments.

Sediment methane concentrations below the surface maxima also varied with distance from shore. On the upper continental shelf, methane levels in this layer generally ranged from 15 to 20 μℓ/ℓ pore water and showed no trends with depth in the upper 1.5 m of sediment. In the slope region, concentrations decreased progressively in an offshore direction to less than 5 μℓ methane /ℓ pore water, and slight increases in methane concentration were observed with depth in the sediment. Higher methane concentrations in the shelf were attributed to greater microbial activity in the sediments as a result of higher temperatures and greater amounts of available organic material than in sediments deposited further offshore.

Concentrations of the non-methane LMWH (ethene, ethane, propene, and propane) at Station 50 are plotted against sediment depth in Figure 3.47. These profiles were representative of the concentrations measured in continental shelf and slope sediments and illustrated the behavior of the light hydrocarbons in the top 1.5 m in this region.

Ethene concentrations fluctuated with depth in the cores, whereas ethane, propene, and propane were relatively constant. Ethene levels were typically twice as high as the other gases, but no trends with sediment depth were observed. Figure 3.48 shows average concentrations of the four hydrocarbons throughout the cores of Transect I stations (Figure 3.45). The average concentration of each hydrocarbon was highest nearshore and decreased seaward until fairly uniform values were observed in the continental slope region (Station 49, 50 and 51). Average concentrations along Transect II followed the same trend although no samples were taken in the slope region for comparison.

The trends of the C_2 and C_3 hydrocarbons with distance from shore were similar to the behavior of methane. These patterns suggested that the concentrations of the C_2 and C_3 in the top few meters of shelf and slope sediments were microbially supported. Like methane, concentrations of the C_2 and C_3 hydrocarbons were probably controlled by biological oxidation and diffusion into the overlying water.

The concentrations of the hydrocarbons listed in Appendix B, Table 25 generally represent "baseline values" in the Texas shelf region. However, anomalous concentrations of ethane and propane were observed in one area of the shelf. Anomalous hydrocarbon concentrations were seen in near-

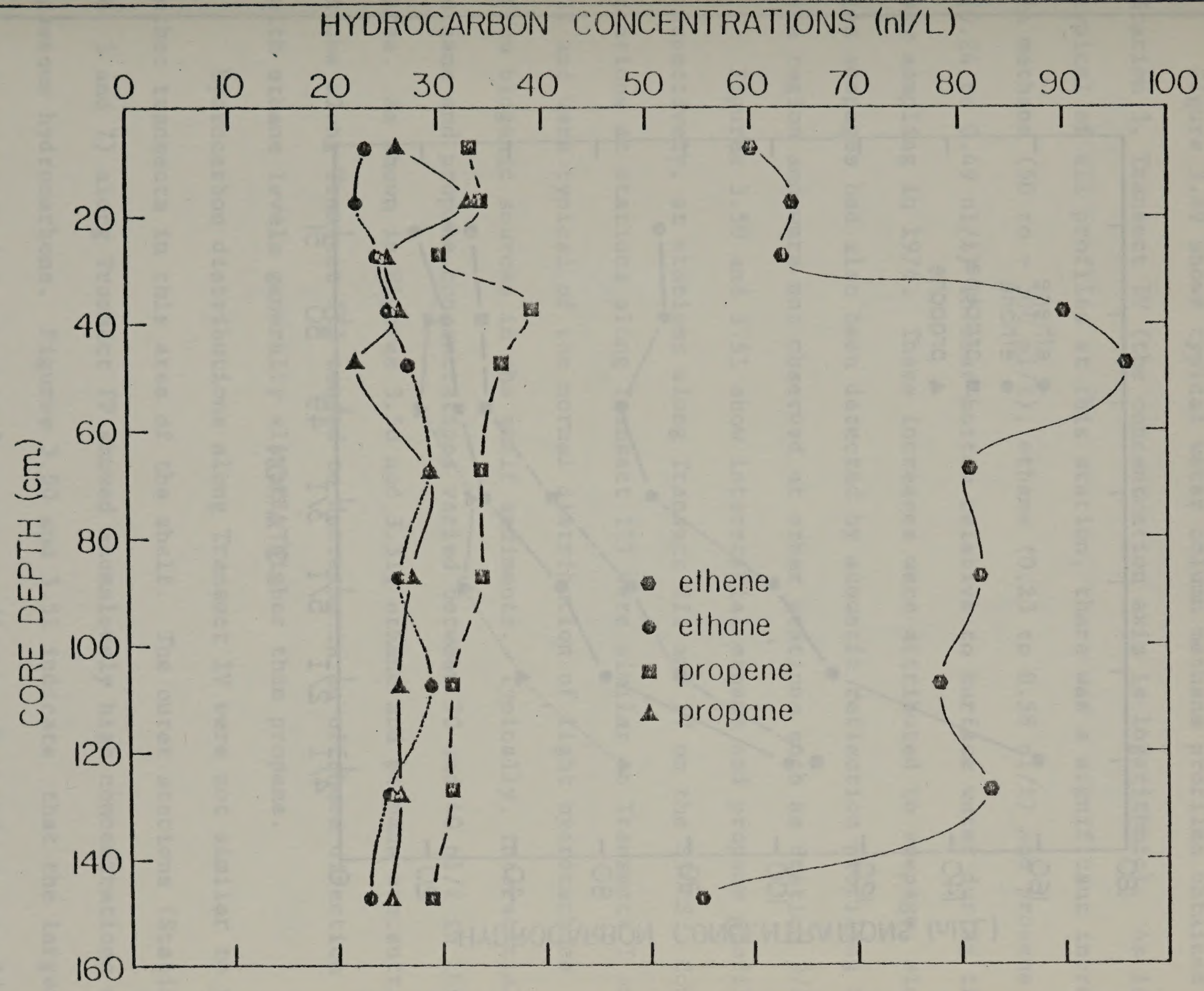

Figure 3.47 Interstitial Concentrations of the C_2 and C_3 Hydrocarbons at Transect I Stations.

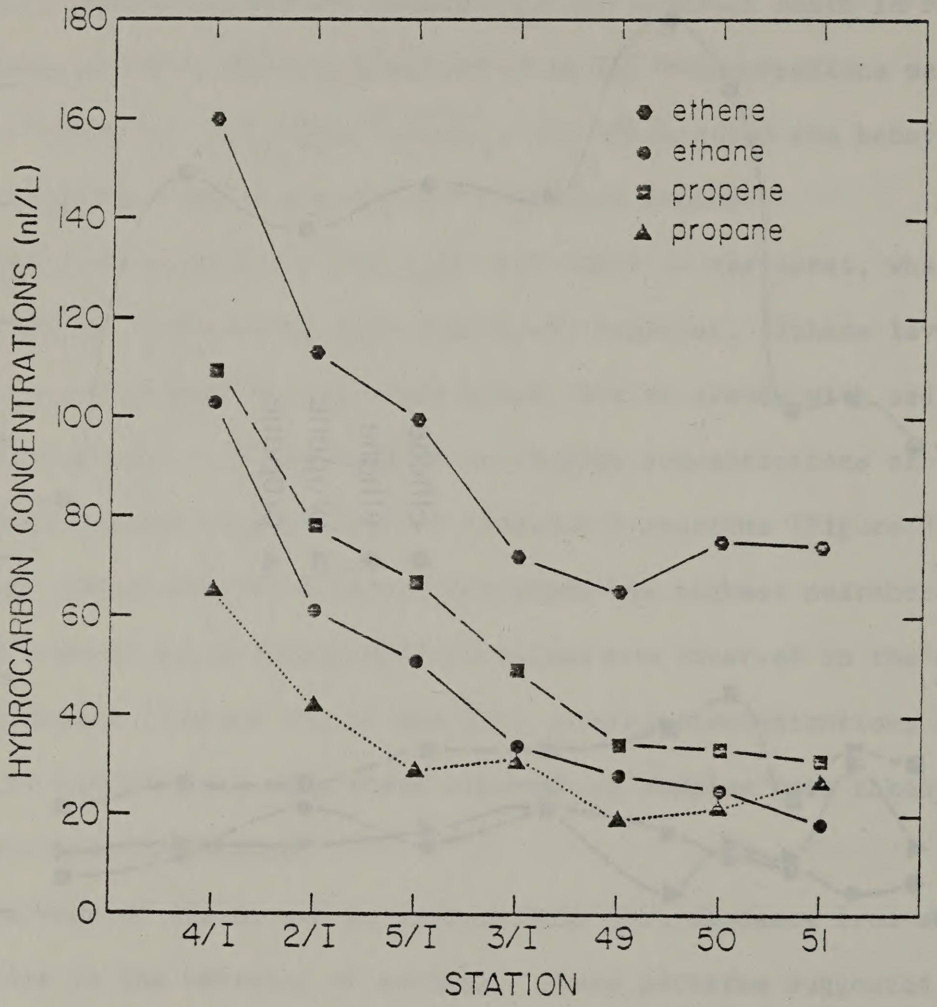

Figure 3.48 Average Concentrations of the C_2 and C_3 Hydrocarbons at Transect I Stations.

bottom water for all nine samplings of Stations 3/IV and sediments at Stations 6, 3, and 7, Transect IV, indicating the presence of anomalous seepage in the region.

Figure 3.49 shows typical water column methane profiles obtained at Station 3, Transect IV (the concentration axis is logarithmic). As is typical of all profiles at this station, there was a significant increase in methane (50 to ~ 500 nl/ℓ), ethane (0.23 to 0.58 nl/ℓ) and propane (0.24 to 0.49 nl/ℓ) in near-bottom relative to surface water during the May sampling in 1976. These increases were attributed to seepage, since gas seepages had also been detected by acoustic reflection profiling in the region and were not observed at other stations such as Station 3/III.

Figures 3.50 and 3.51 show interstitial ethane and propane profiles, respectively, at stations along Transect III and IV on the STOCS. Concentrations at stations along Transect III were similar to Transects I and II and were typical of the normal distribution of light hydrocarbons from biogenic sources in the shelf sediments. Typically, interstitial ethane and propane concentrations varied between 20 and 40 nl/ℓ in this area. As shown in Figures 3.50 and 3.51, ethane and propane concentrations along Transect III tended to decrease in an offshore direction with ethane levels generally slightly higher than propane.

Hydrocarbon distributions along Transect IV were not similar to the other transects in this area of the shelf. The outer stations (Stations 6, 3 and 7) along Transect IV showed anomalously high concentrations of gaseous hydrocarbons. Figures 3.50 and 3.51 indicate that the largest anomalies occurred at Station 3, corresponding to observed seepage identified by near-bottom water sampling. The interstitial ethane and propane concentrations were at least two orders of magnitude above normal

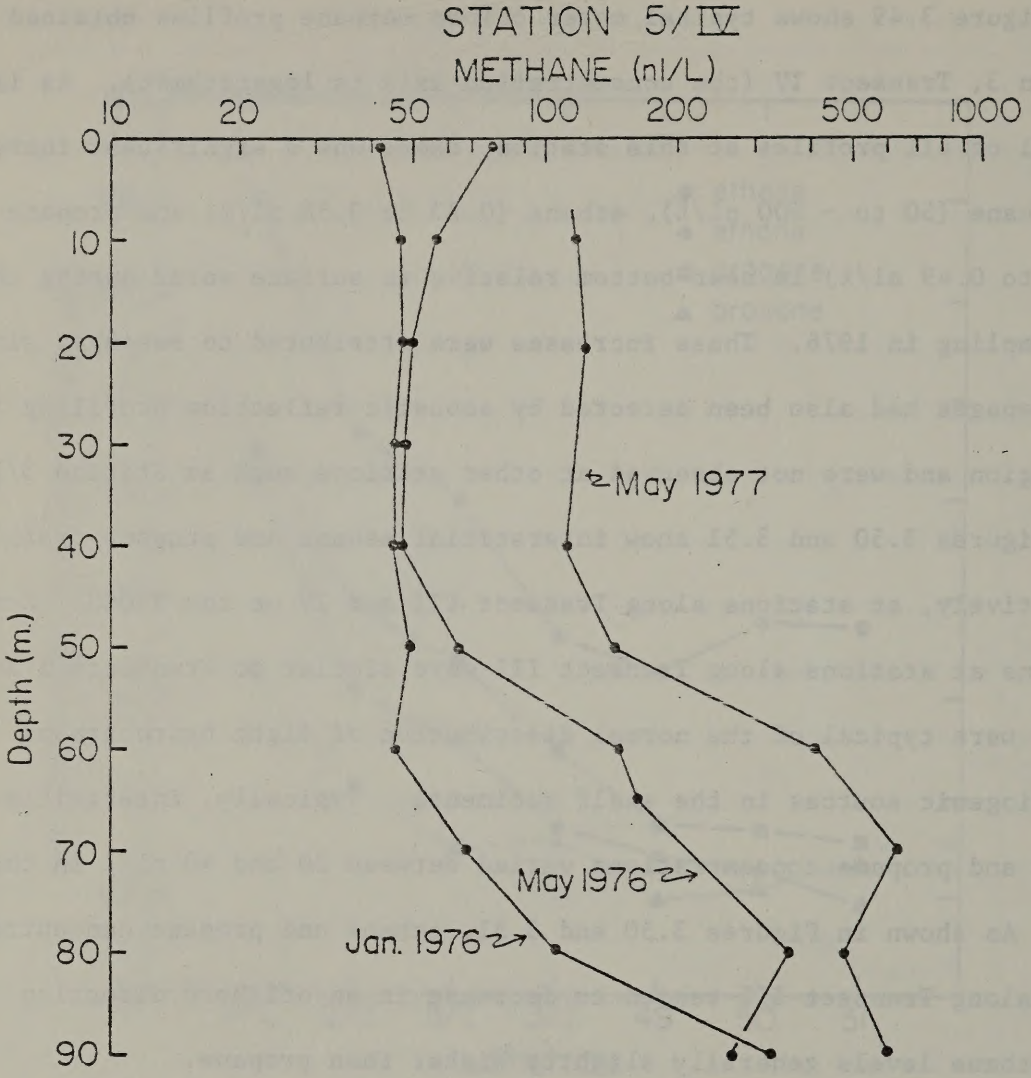

Figure 3.49 Three Water Column Methane Profiles Illustrating Bottom Seepage at Station 3, Transect IV.

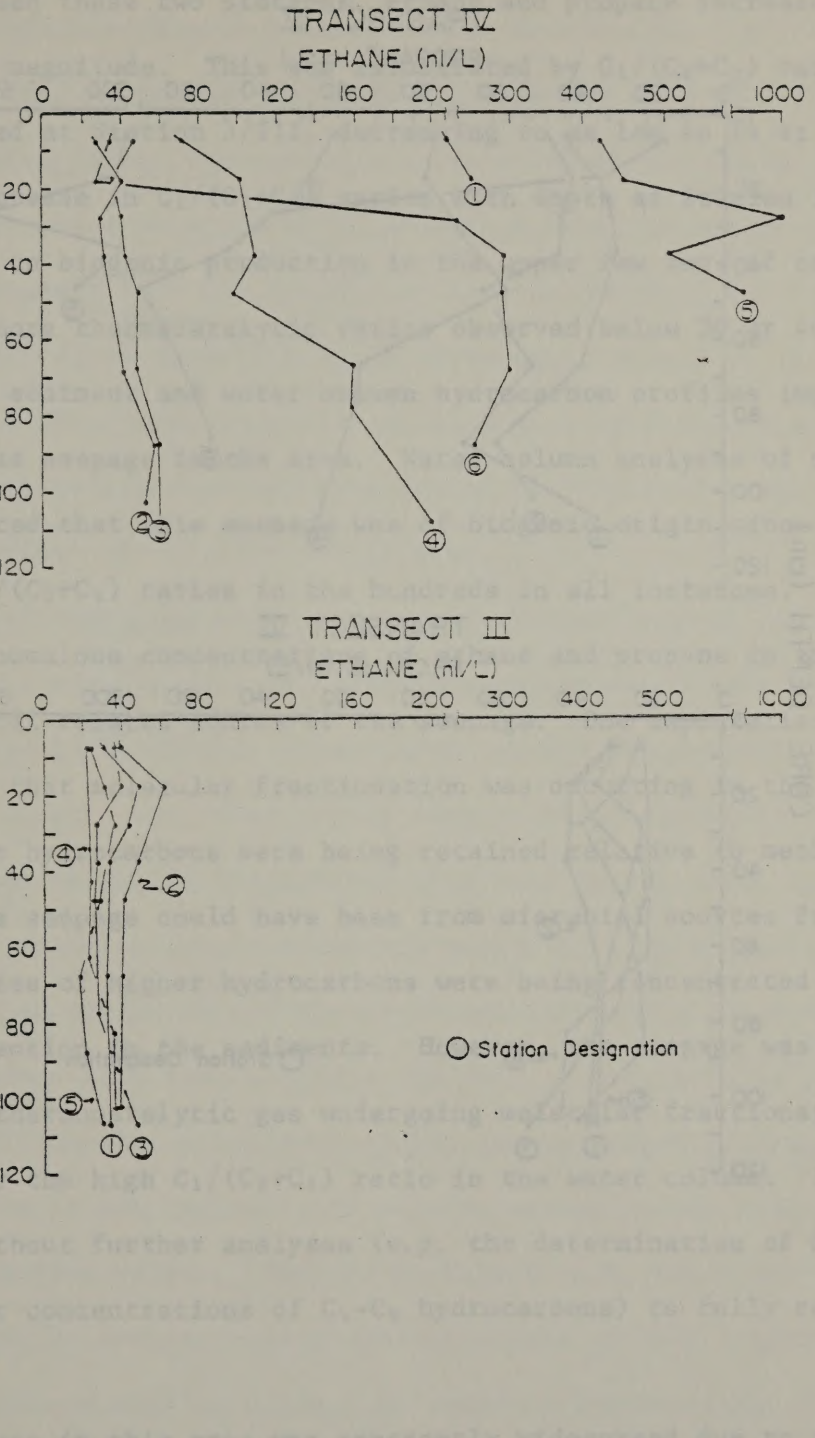

Figure 3.50 Interstitial Ethane Concentrations (Nannoliter per liter pore water) at Stations Along Transects III and IV.

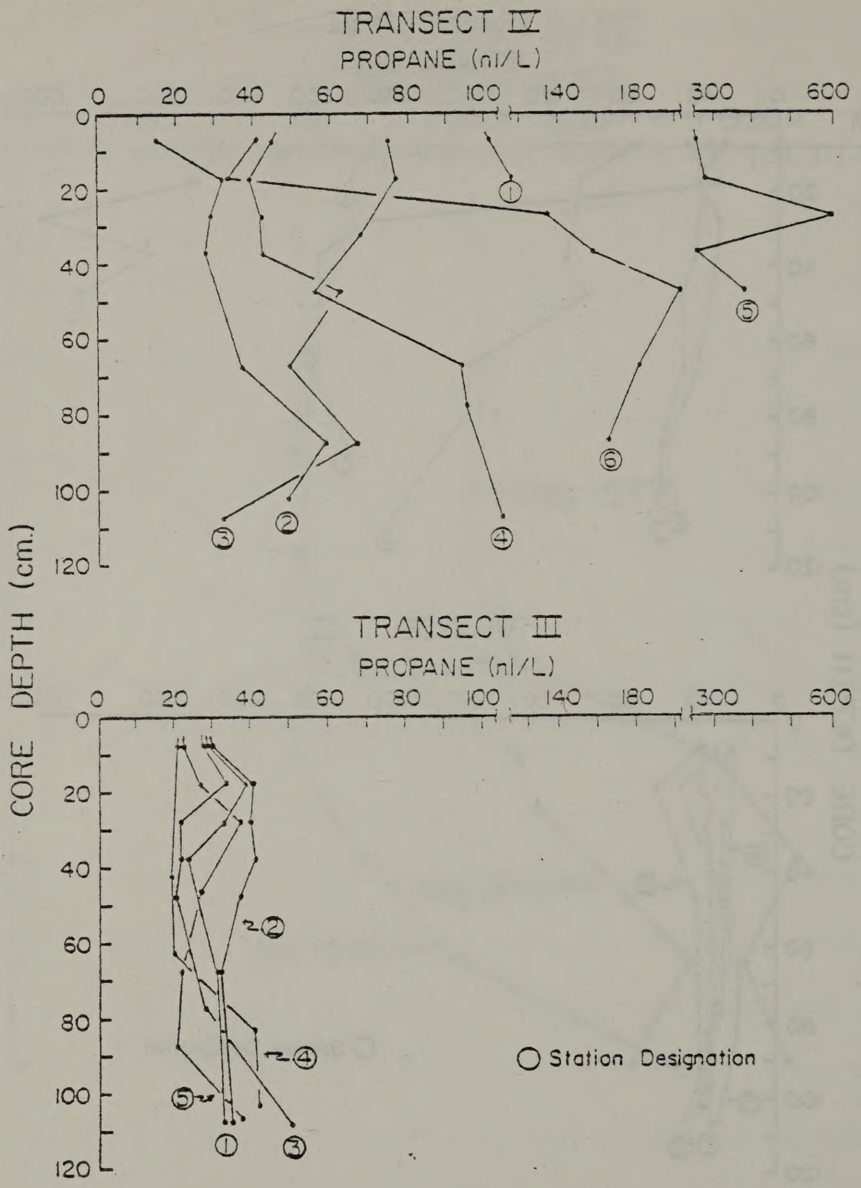

Figure 3.51 Interstitial Propane Concentrations (Nannoliter per liter pore water) at Stations along Transects III and IV.

levels in the outer three stations. Figure 3.52 shows C_1-C_3 hydrocarbon concentrations and $C_1/(C_2+C_3)$ ratios in sediments at Stations 3/IV and 3/III. Although interstitial methane increased from 10 to greater than 30 μℓ/ℓ between these two stations, ethane and propane increased almost two orders of magnitude. This was illustrated by $C_1/(C_2+C_3)$ ratios of several hundred at Station 3/III, decreasing to as low as 14 at Station 3/IV. The decrease in $C_1/(C_2+C_3)$ ratios with depth at Station 3/IV could be attributed to biogenic production in the upper few tens of centimeters diluting the more thermocatalytic ratios observed below 30 or 40 cm.

Both the sediment and water column hydrocarbon profiles implied the presence of gas seepage in the area. Water column analyses of near-bottom waters suggested that this seepage was of biogenic origin since these waters had $C_1/(C_2+C_3)$ ratios in the hundreds in all instances. On the other hand, anomalous concentrations of ethane and propane in the sediments suggested an oil-related source of the seepage. One explanation for this dichotomy was that molecular fractionation was occurring in the sediments, so that higher hydrocarbons were being retained relative to methane. In this case, the seepage could have been from microbial sources from which small quantities of higher hydrocarbons were being concentrated by preferential retention in the sediments. However, the seepage was more likely to be thermocatalytic gas undergoing molecular fractionation, accounting for the high $C_1/(C_2+C_3)$ ratio in the water column. It was impossible without further analyses (*e.g.* the determination of $\delta^{13}C$ of the methane or concentrations of C_4-C_6 hydrocarbons) to fully resolve this problem.

The seepage in this area was apparently widespread due to the spatial extent of the anomalous concentrations both in near-bottom waters and sediments. It appeared that there was both molecular and discrete bubble

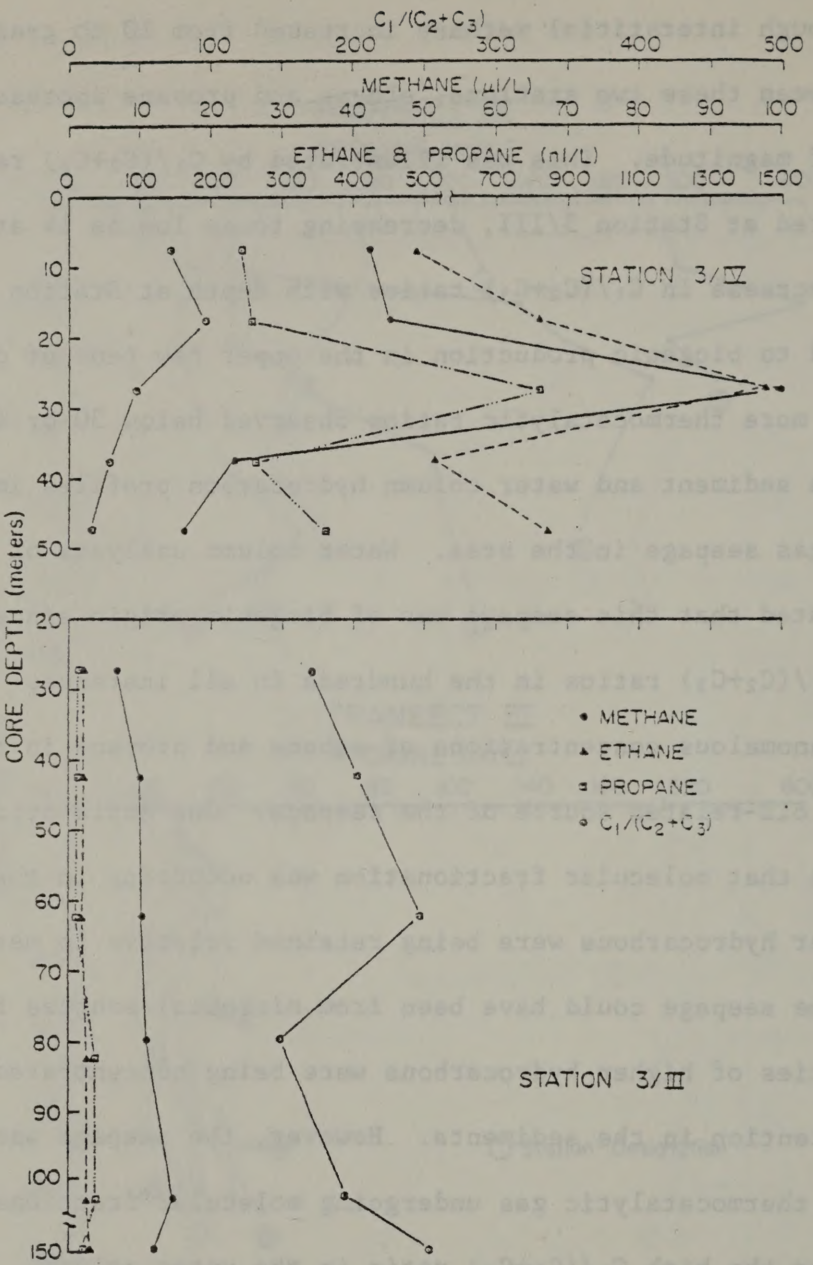

Figure 3.52 Interstitial Hydrocarbon Concentrations (Nannoliters per liter pore water) at Shelf Break Stations on Transects III and IV.

seepage of gas in this area. Ignoring the fact that bubbling seeps had been observed acoustically in this region, one would have predicted that there was bubbling seepage occurring since hydrocarbon anomalies were seen in most instances almost 50 m above the bottom and, for several profiles, all of the way to the sea surface. Bubbling seepage would have contributed hydrocarbons throughout the water column as bubbles rose, although the greatest solution would have been near the bottom due to hydrostatic pressure. If the seepage were only occurring due to molecular diffusion, hydrocarbon anomalies would have been observed in the very bottom waters. Therefore, molecular diffusion could not have accounted for the large anomalies observed considering the rapid movement of water through this relatively shallow (90 m) area.

Hydrocarbon anomalies were observed in the sediment at all three outer stations along Transect IV (ranging in water depth from 65 to 130 m). It would be fortuitous if three cores taken at random were actually over bubbling seeps. Therefore, there must be some mechanism by which the seeping hydrocarbon gas is distributed over this fairly large area. The concentrations measured in the sediments were most likely controlled in part by molecular diffusion but the exact mechanism by which subsurface reservoirs produce high concentrations in surface sediments is poorly understood. Presumably, petroleum-related gas had migrated upward through natural conduits in the sediment and was dispersing in the near-surface sediments and bottom waters. Therefore, future work should include attempts to core on distinct topographic features in this region, such as faults, fractures, or bedding planes, in order to locate sites of distinct petroleum-related gas seepage.

CONCLUSIONS

The STOCS is relatively "clean" with respect to hydrocarbons, as LMWH in the area are chiefly derived from natural sources. The major source of methane appeared to be *in situ* production in the water column. There appeared to be a seasonal pattern to the vertical distribution of methane in the water column. In the winter, due to turbulent mixing, the water column was fairly uniform with respect to saturated LMWH. During the summer and fall, as stratification of the water column developed, a maximum in methane associated with the thermocline developed. This concentration maximum could be almost an order of magnitude higher than levels above and below. The maximum probably resulted from accumulation of suspended matter on the stratification boundary due to restriction of settling velocities across the density gradient, with subsequent production of methane in small micro-reducing environments of suspended particles.

The unsaturated hydrocarbons (*e.g.* ethene and propene) generally follow productivity patterns, being low in winter with higher values in the spring, summer and fall. Ethene and propene were known to be produced by biological processes, thus their strong correlations with phytoplankton productivity parameters were to be expected. Ethene also showed a shallow subsurface maximum associated with a productivity maximum. The unsaturates dominated over their saturated analogs in the STOCS area.

Oxygen concentrations in the upper 60 m of the STOCS region varied seasonally, being generally highest at nearshore stations in the winter and lowest in the summer. Temperature and salinities were measured on subsamples of water taken for oxygen determinations so that equilibrium oxygen concentrations could be calculated and compared to measured values. Ratios of measured oxygen to equilibrium oxygen concentrations indicated that oxygen variations in the upper 60 m were generally controlled by physical processes (seasonal changes in seawater temperature and salinity) rather than productivity fluctuations. The mass of highly oxygenated water could be traced by cross-sectional concentration contours as it was formed nearshore in the winter and displaced by warming in the spring and summer. The intrusion of oxygen-depleted 200-300 m Western Gulf Water was evident year-round below approximately 70 m in the STOCS region, and seasonal variations in stratification of the water column could be seen by the extent of vertical mixing with this bottom water.

Nutrient concentrations were representative of open Gulf surface water in most of the water above 60 m depth, but continental run-off influenced nearshore concentrations, especially in the spring. Nitrate as the limiting nutrient to productivity disappeared after the spring phytoplankton blooms through the summer and early fall. Phosphate and silicate were affected by the high spring productivity but not completely removed. These nutrients were gradually replenished during the summer and fall to moderately high values by December. The intrusion of the nutrient-rich 200-300 m Western Gulf Water could again be clearly seen below 70 m from nutrient concentration contours across cross-sectional diagrams of Transect II.

Concentrations of light hydrocarbons in the top few meters of Texas continental shelf and slope sediments were highest near shore and decreased regularly in an offshore direction. Vertical methane profiles exhibited maxima in the top 40 cm of sediment on the shelf, in contrast to downward-increasing gradients in the slope region.

Methane was apparently microbially produced in micro-reducing environments and removed by biological oxidation and diffusion into the overlying water. Production rates were related to microbial activity, organic content, and temperature of the sediments. Profiles of C_2 and C_3 hydrocarbons implied that background concentrations of these gases were also controlled by microbial processes.

These conclusions implied that methane concentrations in near-surface shelf sediments were seasonally influenced. Warmer temperatures and increased detrital and nutrient input in the spring might have enhanced microbial production, so that oscillations of methane could be observed. If real, these oscillations in methane concentration could provide an excellent means of quantitating the magnitude of the effective diffusion coefficient of methane in porous sediments.

Interstitial concentrations of ethene, ethane, propene, and propane were relatively constant with depth in the upper two meters of shelf, slope, and abyssal sediments, decreasing progressively from 160, 100, 110 and 60 nl/ℓ pore water in nearshore sediments, to fairly uniform levels of 80, 25, 30 and 25 nl/ℓ downslope, respectively. The concentrations reported here generally represent "baseline values" of the light hydrocarbons on the Texas shelf. One area of anomalously high ethane and propane was found, however, indicating an input of thermocatalytic gas from the subsurface. This gas was apparently migrating upward through natural conduits in the sediment and dispersing in near-surface sediments and bottom waters in the region. Future sediment work as well as prospecting for reservoired hydrocarbons should include coring on or near geological features such as unconformities, faults, bedding planes, or distinct gas seepage in an effort to detect additional anomalous gas zones.

LITERATURE CITED

Atkinson, L. P. and F. A. Richards. 1967. The occurrence and distribution of CH_4 in the marine environment. Deep Sea Res. 14:693.

Barnes, R. O. and E. D. Goldburg. 1976. Methane production and consumption in anoxic marine sediments. Geology 4:297-300.

Bernard, B. B., J. M. Brooks and W. M. Sackett. In Press. Light hydrocarbons in recent Texas shelf and slope sediments. J. Geophys. Res.

____________. 1976. Natural gas seepage in the Gulf of Mexico. Earth. Planet. Sci. Lett. 31:48.

Broecker, W. B. Interstitial water studies, Leg 15 - Introduction and summary. pp 751-755. *In* Supko, P. R. (ed.), Initial reports of the deep sea drilling project XX, U. S. Government Printing Office, Washington, D. C.

Brooks, J. M. 1976. The flux of light hydrocarbons into the Gulf of Mexico via runoff. *In* Marine Pollutant Transfer, H. L. Windom and R. A. Duce (eds.) D. E. Heath and Co., Lexington, Mass.

________. 1975. Sources, sinks, concentrations and sub-lethal effects of light aliphatic and aromatic hydrocarbons in the Gulf of Mexico. Ph.D. Dissertation, Texas A&M University.

________., B. B. Bernard, and T. C. Sauer. In Press. Environmental aspects of a well blowout in the Gulf of Mexico. Environmental Sci. Technol.

________., B. B. Bernard, and W. M. Sackett. 1977. Input of low-molecular-weight hydrocarbons from petroleum operations into the Gulf of Mexico. *In* Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms.

________., and W. M. Sackett. In Press (1977). Significance of low-molecular-weight hydrocarbons in coastal waters. *In* Advances in organic geochemistry, 1975.

________., J. R. Gormly, and W. M. Sackett. 1974. Molecular and isotopic composition of two seep gases from the Gulf of Mexico. Geophys. Res. Lett. 1:213.

________., A. D. Fredricks, W. M. Sackett, and J. W. Swinnerton. 1973. Baseline concentrations of light hydrocarbons in the Gulf of Mexico. Environm. Sci. Technol. 7:639.

________., and W. M. Sackett. 1973. Sources, sinks, and concentrations of light hydrocarbons in the Gulf of Mexico. J. Geophys. Res. 78:5248.

Cappenburg, T. E. 1975. A study of mixed continuous cultures of sulfate-reducing and methane-producing bacteria. Microbial. Ecol. 2:60-72.

________., 1974a. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake I. Field Observations. Antonic van Leeuwenhoek 40:285-295.

________., 1974b. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake II. Inhibition Experiments. Antonic van Leeuwenhoek, 40:297-306.

________., and H. Prins. 1974. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. III. Experiments with ^{14}C-labeled substrates. Antonic van Leeubenhoek, 40:457-469.

Certes, A. 1884. Sur la culture, a l' abri des germes atmospheriques, des eaux et des sediments rapports par less expeditions due Travailleur et du Talisman. Compt. Rend. Acad. Sci. 98:690-693.

Claypool, G. E., and I. R. Kaplan. 1974. The origin and distribution of Methane in marine sediments, pp 99-139. *In* Natural gases in marine sediments, I. R. Kaplan (ed.), Plenum, New York.

Coleman, D. D. 1976. Isotopic composition of Illinois Natural gas. Ph.D. Dissertation, Univ. of Illinois, Urbana-Champaign. 175pp.

Davis, J. B., and H. F. Yarbrough. 1966. Anaerobic oxidation of hydrocarbons by *Desulfovibrio desulfuricans*. Chem. Geol. 1:137-144.

________., and R. M. Squires. 1954. Detection of microbially produced gaseous hydrocarbons other than methane. Sci. 119:381-382.

Drew, G. H. 1912. Report of investigations on marine bacteria carried on at Andros Island, Bahamas, British West Indies, in May 1912. Year-book Carnegie Inst. 1:136.

Jannasch, W. H., K. Eimhjellen, C. O. Wilsen, and A. Farmantaraian. 1971. Microbial degradation of organic matter in the deep sea. Sci. 171:672-67

Kaplan, I. R., and S. C. Rittenburg. 1963. Basin sedimentation and diagenesis in the sea. The Earth beneath the sea, history. 3:583-619.

Lamontagne, R. A., J. W. Swinnerton, and V. J. Linnenbom. 1974. C_1-C_4 hydrocarbons in the North and South Pacific. Tellus 26:71.

____________., and W. Smith. 1973. Methane concentrations in various marine environments., J. Geophys. Res. 78:5317.

____________., 1971. Nonequilibrium of carbon monoxide and methane at the air-sea interface. J. Geophys. Res. 76:5117.

Lloyd, B. 1931. Muds of the Clyde Sea area II. Bacterial content. J. Mar. Biol. Assoc. 17:751-765.

MacDonald, R. W. 1976. Distribution of low-molecular-weight hydrocarbons in Southern Beaufort Sea. Environ. Sci. Technol. 10:1241.

McAullife, C. 1971. Gas chromatographic determination of solutes by multiple phase equilibrium. Chem. Technol. 1:46.

________., 1966. Solubility in water of paraffin, cycloparaffin, olefin, acetylene, cycloolefin, and aromatic hydrocarbons. J. Phys. Chem. 70:1267-1275.

Martens, C. S. and R. A. Berner. 1977. Interstitial water chemistry of anoxic Long Island Sound sediments I. Dissolved gases. Limnol. Oceanogr., 22:10-25.

____________., 1974. Methane production in the interstitial waters of sulfate-depleted marine sediments. Sci. 185:1167-1169.

Oremland, R. S., and B. F. Taylor. 1978. Sulfate reduction and methanogenesis in marine sediments. Geochim. et. Cosmochim. Acta. 42:209-214.

Presley, B. J., M. B. Goldhaber, and I. R. Kaplan. 1970. Interstitial water chemistry, deep sea drilling project, Leg 5., *In* Initial reports of the deep sea drilling project, V. T. A. Davies (ed.) U.S. Government Printing Office, Washington, D. C. pp 513-522.

Quayle, J. R. 1972. The metabolism of one-carbon compounds by microorganisms. Adv. Microb. Physiol. 7:119-203.

Reeburgh, W. S. 1976. Methane consumption in Cariaco Trench waters and sediments. Earth Planet. Sci. Lett. 28:337-344.

________., and D. T. Heggie. 1977. Microbial methane consumption reactions and their effect on methane distributions in freshwater and marine systems. Limnol. Oceanogr. 22:1-9.

Reuszer, H. W. 1933. Marine bacteria and their role in the cycle of life in the sea. III. Distribution of bacteria in the open ocean waters and muds about Cape Cod, Biol. Bull. 65:480-497.

Russell, H. L. 1892. Untersuchungen uber im Golf von Neapel lebende Bakterien. Zeitschr. f. Hyg., 2:165-206.

Sackett, W. M. and J. M. Brooks. 1975. Origin and distribution of low-molecular weight hydrocarbons in Gulf of Mexico coastal waters. *In* Marine chemistry in the coastal environment, Church, T. M. (ed.) ACS Symposium Series 18, ACS Washington pp 211-230.

____________. 1974. The use of low-molecular-weight hydrocarbon concentrations as indicators of marine pollution, NBS Spec. Publ. 409 Marine Pollution Monitoring (Petroleum), Proceedings of a Symposium and Workshop held at NBS, Gaithersburg, Maryland, May 13-17.

Scranton, M. I., and P. G. Brewer. In Press. Occurrence of methane in the near-surface waters of the Western Subtropical North Atlantic. Deep Sea Res.

Seiler, W., and V. Schmidt. 1974. Dissolved non-conservative gases in sea water. *In* The sea, vol. V., Goldberg, E. D. (ed.), Wiley Interscience, New York.

Sorokin, Y. I. 1957. Ability of sulfate reducing bacteria to utilize methane for education of sulfates to hydrogen sulfide. Mikrobiologiya, 115:816-818.

Strickland, J. D. H., and T. R. Parsons. 1972. A practial handbook of seawater analysis. Bulleton 167, Fish. Res. Bd. Canada, Ottawa.

Swinnerton, J. W., and R. A. Lamontagne. 1974. Oceanic distribution of low-molecular-weight hydrocarbons: baseline measurements. Environ. Sci. Technol. 8:657.

_________., V. J. Linnenbom, and C. H. Cheek. 1969. Distribution of methane and carbon monoxide between the atmosphere and natural waters. Environ. Sci. Technol. 3:838.

_________., and V. J. Linnenbom. 1967. Gaseous hydrocarbons in seawater: determination. Sci. 156:1119.

Tinkle, A. R., J. W. Antoine, and R. Kuzeia. 1973. Detecting natural gas seeps at sea. Ocean Industry 8:139.

Weiss, R. F. 1970. The solubility of nitrogen, oxygen, and argon in water and seawater. Deep-Sea Res. 17:721.

Yamamoto, S., J. B. Alcauskas, and T. E. Crozier. 1976. Solubility of emthane in distilled water and seawater. J. Chem. Eng. Data 21:78.

Zobell, C. E. 1946. Marine microbiology. Chronica Botanica Co., Waltham, Mass. 240 pp.

_________., 1942. Changes produced by microorganisms in sediments after deposition. J. Sed. Petrology, 12:127-136.

_________., and D. Q. Anderson. 1936. Vertical distribution of bacteria in marine sediments, Bulletin of the American Assoc. of Petroleum Geologists, 20:258-269.

CHAPTER FOUR

HIGH-MOLECULAR-WEIGHT HYDROCARBONS IN ZOOPLANKTON, SEDIMENT AND WATER

TOTAL ORGANIC CARBON AND DELTA C^{13} IN SEDIMENT

SUMMARY OF HIGH-MOLECULAR-WEIGHT HYDROCARBON ANALYSES CARRIED OUT IN SUPPORT OF THE MICROBIOLOGY PROJECT AND THE TOPO-HIGH PROGRAM

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ABSTRACT

An extensive survey of the level of natural and petroleum type hydrocarbons in seawater, zooplankton and sediment from the South Texas Outer Continental Shelf (STOCS) was made. The presence or absence of indicator parameters, including n-paraffins, odd-even ratios, unresolved GLC humps, and GC/MS confirmation of aromatic hydrocarbons, were taken as a measure of petroleum contamination.

Some zooplankton samples showed contamination with petroleum-like hydrocarbons. Twenty-eight (28) of the 60 samples showed contamination. Similar studies in 1975 and 1976 showed approximately 7 and 30% contamination respectively. This apparent increase in contamination may have resulted from increased transportation of petroleum and petroleum products through the area.

Dissolved and particulate hydrocarbons in seawater were determined in 56 samples. Concentrations of dissolved and particulate hydrocarbons were similar in magnitude. Particulate hydrocarbon concentration generally decreased with distance offshore; dissolved hydrocarbon concentration showed less variation. Winter and spring samples of both fractions had higher average concentrations than did fall samples. The hydrocarbon composition of dissolved and particulate samples were similar. The most abundant n-alkanes were in the $C_{27}-C_{33}$ range with a slight preference for odd carbon numbers.

Hydrocarbon analyses of 15 sediment samples from the open shelf showed that the sedimentary environment was still a pristine one. No gross contamination of sediments with petroleum-like hydrocarbons has been observed. Total organic carbon content increased with increasing distance from shore, probably reflecting the increasing clay content of the sediments. Stable carbon isotope distributions were fairly uniform throughout the STOCS area. A possible trend of more positive Delta C^{13} values shoreward may have been due inclusion of estuarine derived organic matter (seagrasses) in these sediments.

INTRODUCTION

The level of petroleum derived organic compounds in the marine environment relates to two questions which differ only in reference to their respective time-frames. First, it must be established whether present day levels of oil in the sea are having a deleterious effect on plants and animals. Second, it is necessary to establish the long term trends of pollutant levels so that the eventual consequence of the utilization of non-renewable or living resources can be predicted.

The studies reported here provide a partial answer to these two questions. By combining environmental concentration data with data on biological effects from other studies, a crude idea of the degree of present day ecological damage can be induced. The second use of environmental data is to serve as a data base against which future levels and patterns of pollution can be compared. While sophisticated statistical inferences cannot be drawn from the present data base, trends and patterns can be recognized to develop hypotheses for future study.

Background

Careful chemical analyses were carried out for a large suite of samples. The approach taken was to isolate the hydrocarbon fraction from material from the STOCS area and to identify molecules which are the common components of petroleum. This report deals with hydrocarbons in the molecular weight range of C-15 to C-36, saturated, unsaturated and aromatics isolated from the water column, zooplankton tows and sediments. In addition measurements of Delta C^{13} were made to evaluate the potential of using this parameter to recognize petroleum derived carbon in sediment.

The hydrocarbon geochemistry of seawater, plankton and sediment are best understood when viewed as part of the overall organic geochemistry of the ecosystem. A very simple model of the carbon cycle in coastal waters is useful for pointing to critical relations. Figure 4.1 illustrates such a model. All organic matter in the system is derived from the photosynthetic fixation of inorganic carbon by phytoplankton. Hydrocarbons are among the natural products which make up each of the carbon reservoirs shown. However, as Tables 4.1, 4.2 and 4.3 show, hydrocarbons are minor components. The hydrocarbons synthesized by plants, animals and bacteria and modified by chemical reactions are in general fairly simple, such as normal and isoprenoid saturated and unsaturated compounds. Aromatic hydrocarbons and most of the hetero-compounds characteristic of petroleum are absent. It is this organic geochemical generalization that has guided the experimental design of the STOCS baseline study of hydrocarbons.

MATERIALS AND METHODS

The goal of this project was to obtain a significant body of analytical data on environmental hydrocarbon levels and Delta C^{13} values which could serve as a data base against which future levels might be compared. An intense effort was made to identify hydrocarbon molecules which might serve as indicators of petroleum. The experimental elements were careful collection, preservation and transportation of samples in the field; laboratory analyses using as nearly as possible standardized and modern techniques; and central data storage and computer analysis. A study of the level of total organic matter in sediments was done to provide nutritional baseline data for the benthic biology program.

Sample Collection

The sampling frequency, stations sampled, and number of samples col-

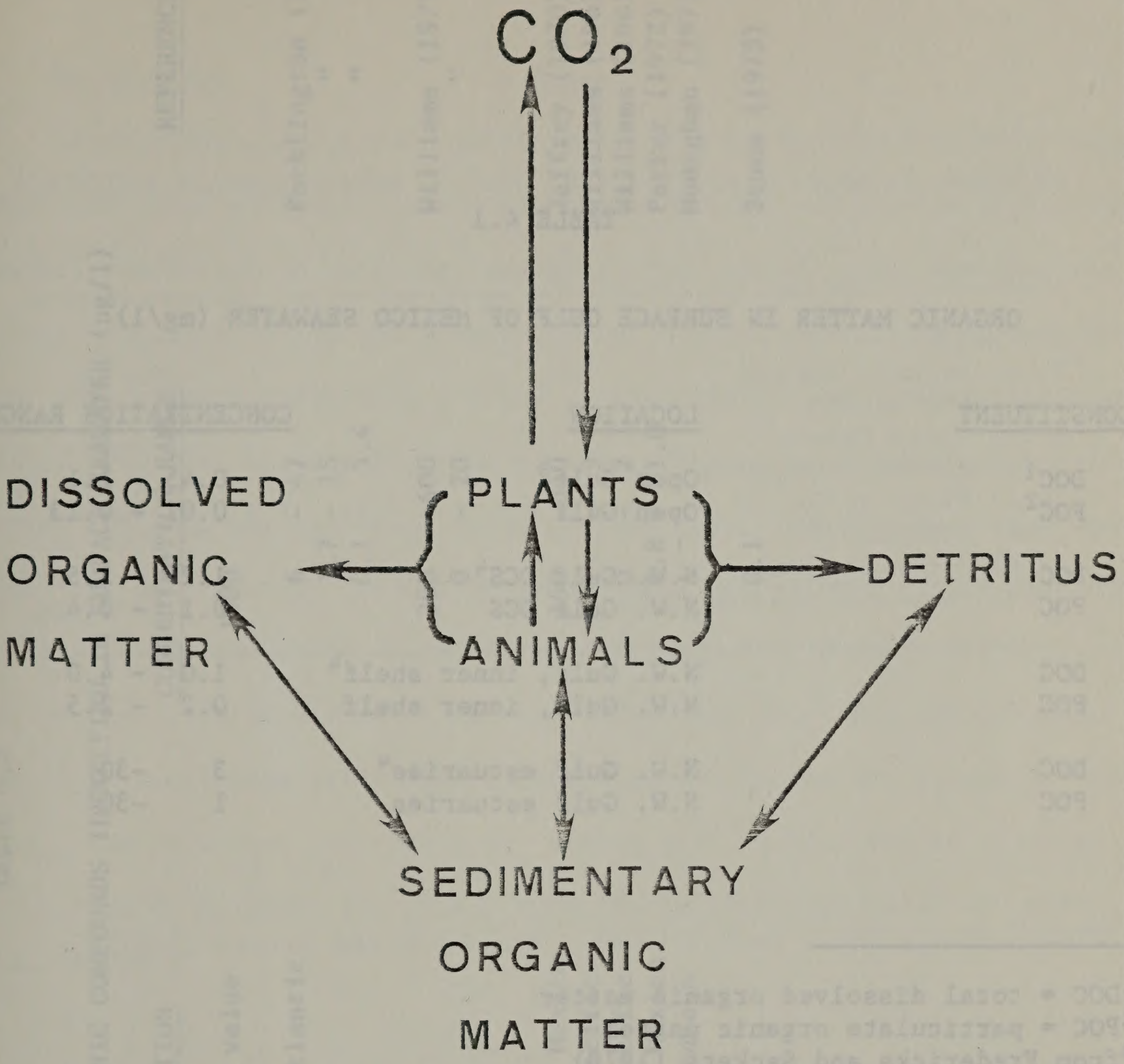

Figure 4.1 Model of the Carbon Cycle in Coastal Waters.

TABLE 4.1

ORGANIC MATTER IN SURFACE GULF OF MEXICO SEAWATER (mg/l)

CONSTITUENT	LOCATION	CONCENTRATION RANGE
DOC[1]	Open Gulf[3]	0.45 - 1.1
POC[2]	Open Gulf	0.02 - 0.13
DOC	N.W. Gulf OCS[4]	1.2 - 2.5
POC	N.W. Gulf OCS	0.1 - 0.4
DOC	N.W. Gulf, inner shelf[4]	1.0 - 4.0
POC	N.W. Gulf, inner shelf	0.2 - 2.5
DOC	N.W. Gulf estuaries[4]	3 -30
POC	N.W. Gulf estuaries	1 -30

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[1] DOC = total dissolved organic matter
[2] POC = particulate organic matter
[3] from Fredericks and Sackett (1970)
[4] from Maurer and Parker (1972)

TABLE 4.2

SPECIFIC DISSOLVED ORGANIC COMPOUNDS IDENTIFIED IN SURFACE SEAWATER (μg/l)

SUBSTANCE	LOCATION	CONCENTRATION RANGE	REFERENCES
DOC[1]	Average value	1000	
Amino Acids total	North Atlantic	6 - 47	Pocklington (1971)
Alanine	"	1.7 - 15	"
Leucine	"	0.1 - 5.4	"
Total carbohydrates	Average	200 -600	Williams (1975)
Individual sugars	Average	0 - 20	"
Lipids			
Total Lipids	Gulf of Mexico	150 -310	Jeffrey (1970)
Total Fatty Acids	N. E. Pacific	1 - 9	Williams (1965)
Individual Fatty Acids	N. E. Pacific	0 - 2	Williams (1965)
Total n-Paraffins	Gulf of Mexico	0.8 - 1.0	Parker (1972)
Non-Volatile Hydrocarbons	Mediterranean	4	Monaghan (1973)
Vitamins	Average	0.1	Stumm (1975)

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[1] DOC = total dissolved organic matter

TABLE 4.3

MATERIAL BALANCE IN AN IDEALIZED GULF OF MEXICO SURFACE SEDIMENT[1]

Dry Weight	16 g
Total Organic Carbon	100 mg
Non-Lipid Carbon	95 mg
Total Lipid Carbon	5 mg
Total Non-Saponifiables	3 mg
Total Fatty Acids	0.4 mg
Total Sterols	0.1 mg
Total Fatty Alcohols	0.1 mg
Total Saturated Hydrocarbons	0.03 mg

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[1] from Parker (1967), Parker (1969), and Sever and Parker (1969)

lected was as shown in Table 4.4.

Zooplankton

Zooplankton samples were collected with a 1-m net (250 µm NITEX mesh) which was towed obliquely from near-bottom to near-surface for 15 minutes. The net, dedicated to this use, was kept in a special clean box when not in use. This clean box was constructed of 3/4-in. plywood which was covered inside and outside with a layer of fiberglass cloth sealed with epoxy resin. The box was of sufficient size to contain the frame, net and all lines. The lid was sealed so as to exclude dust. Samples were not "washed down" the net into the cod-end so as to avoid contamination from the ship's pumps. If the net needed cleaning, it was lowered to a depth of 20 m, raised, and lowered without a cod-end collecting jar. The samples were placed in precleaned glass jars with teflon lid liners and frozen on board ship. The jars were precleaned by washing with high phosphate detergent and hot water followed by rinsing with double distilled water and then with methanol.

Seawater

Seawater samples were collected from 10 m below the surface using a collection device which consisted of a glass carboy which could be opened and closed (from the ship's deck) by means of a plug attached to a nylon line. A minimum of 38 ℓ (two, 5-gallon carboys) was taken. The samples were filtered through glass fiber filters which had been precleaned by reflux with chloroform. The nominal pore size of the filters was 1.2 µm. The pads containing the particulate organic matter (POC) were placed in small glass jars with teflon lids and frozen. The filtrate was poisoned with 50 ml of chloroform and processed as soon as possible on returning

TABLE 4.4

SUMMARY OF SAMPLING FREQUENCY, STATIONS SAMPLED AND NUMBER OF SAMPLES

Sample Type	Station/Transect	Time	Number of Samples	
Water/dissolved hc	1-3/I-IV	3 Seasons	36	
Water/dissolved hc	20 replicated taken at random		20	
				56
Water/particulate hc	1-3/I-IV	3 Seasons	36	
Water/particulate hc	20 replicates taken at random		20	
				56
Zooplankton	1-3/I-IV	3 Seasons	36	
	24 replicates taken at random		24	
				60
Sediment/shelf hc	1-3/I-IV	Taken at random	12	
	3 replicates taken at random		3	
				15
Sediment/Topographic Highs hc	1-4/Four banks taken at random		15	
	2-3/East Flower Garden	Spring '78	4	
				19
Sediment/shelf Delta C^{13} and TOC	1-7/I-IV	3 Seasons	75	
	2 + 4/Two banks	3 Seasons	12	
	40 replicates taken at random		40	
				127
Sediment/Topographic Highs, Total Organic Carbon and Delta C^{13}	1-4/Four Banks	1 Season	15	
	2+3/East Flower Garden	Spring '78	2	
				17
Microbiology[1] hc	1-3/II Water Column	9 Monthly	209	
	1-3/I & IV Benthic	3 Seasons	38	
	Special Project Samples	Taken at random	73	
				320

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[1] Received 412 samples but analyzed only 320 as per direction of Program Manager

to the laboratory.

Sediment

Sediment samples were obtained as subsamples of repeated Smith-McIntyre grabs. Each subsample, weighing approximately 10-15 kg, was taken from the top 5 cm of the grab. The samples were placed in precleaned glass jars with teflon lid liners, taking care not to fill each jar more than one-half full. The samples were frozen on board ship and kept frozen until analysis. Obvious marine animals were seldom encountered in the sediment, but were discarded when found. If the sample was a pooled sample, this operation was performed at sea prior to freezing. In cases where chemical analysis was possible within a few days after collection, the samples were maintained at 0°C to avoid the risk of the jar breaking due to freezing.

Laboratory Analysis

Throughout the study, purified solvents, inorganic chemicals and double distilled water were used. Control samples and blanks were used to insure that no gross contamination was present. The laboratory was in a new building and had never been used for any other purposes. At no time was severe contamination encountered. The most serious problem was electronic and background problems in the GC/MS.

Total Organic Carbon and Delta C^{13}

The percent total organic carbon was measured using a high temperature combustion technique which has been used for several years in this laboratory (Hedges and Parker, 1976). The frozen sample, 1-5 g, was thawed and treated with dilute HCl in a tall beaker to remove carbonate carbon. If magnesium or iron carbonate were suspected of being present, the acid was heated to aid decomposition. The mixture was washed with distilled water to remove excess acid. The sediment was collected by filtering onto a glass fiber pad. The damp sediment was removed from the pad, dried at 40°C and ground to pass a 40 mesh sieve. An aliquot of this powder, 100-200 mg was weighed into a LECO clay combustion cup, iron powder and CuO were added and the sample burned in a LECO RF furnace for two minute intervals. The evolved CO_2 was collected by freezing with nitrogen, the excess O_2 was pumped away and the CO_2 measured manometrically (Calder, 1969). The data are expressed as percent organic carbon on a carbonate free basis.

The samples for Delta C^{13} analysis were treated according to the same procedure. In this case, it was necessary to burn a large enough sediment sample to yield three milliliters of CO_2 for mass-spectrometric analysis. The MS analysis was done on a 15.24 cm, 60° sector field mass spectrometer (Model 6-60-RMS-26) made by Nuclide Corp., State College, Pa. The data are reported as permil deviations from the PDB standard. The standard deviation for repeated analyses was 0.3.

Zooplankton Samples

Zooplankton hydrocarbons were isolated and purified using the method which was used in the previous two years of the STOCS program. The samples were quickly thawed by standing the jars in warm water. Care was taken not to contaminate the rim of the jar. The sample was inspected for tar-balls greater than 1 mm and any present were discarded. Micro-tar balls were present in some samples and were taken as a valid part of the sample. The thawed sample (approximately 25 g wet weight) was poured into a pre-cleaned (by extraction) cellulose Soxhlet extraction thimble (Whatman, single thickness, 33 x 80 mm) and allowed to drain. If the seawater filtrate showed color, it was extracted with a few milliliter of toluene which was added to the Soxhlet extractor.

The thimble was placed in a Soxhlet extractor, heated on a steam table, and continuously extracted for 12 hours using a solvent charge of 125 ml of the methanol-toluene (7:3) azeotrope. The extraction was repeated with fresh solvent and the extracts combined. The solid residue in the thimble was dried at 80°C and weighed. The extracts were taken to dryness on a roto-vap at 45°C with one-half an atmosphere of vacuum.

The lipid recovered by Soxhlet extraction was saponified by refluxing (six hours) with a 0.5 KOH-methanol solution. This reflux solution had been pre-purified by extraction with toluene. In cases where GLC indicated that methyl esters were present, the sample was re-saponified. In general, ester formation did not occur if a few milliliters of distilled water were added to the saponification solution an hour prior to reflux termination. When saponification was complete the mixture was transferred while warm to a separatory funnel and enough saturated NaCl solution was added to cause two phases to form when 15 ml of n-hexane was added. The non-saponifiable lipids, which included hydrocarbons, were extracted into three 15 ml portions of n-hexane. The saponifiable fraction was discarded, and the non-saponifiable extracts combined and set aside for purification by column chromatography.

Column chromatography was carried out to purify the total hydrocarbon extract and to separate it into two chemical fractions, saturated hydrocarbons and the non-saturated hydrocarbons, which include both olefins and aromatic molecules. This method is described in the following paragraphs.

A large batch of one part alumina plus two parts silica gel, both Activity I, was prepared using hydrocarbon-free chemicals. Column chromatography was carried out using this material with a sample to packing ratio of 1:300 in a glass column with a teflon stopcock and with a length to inside diameter ratio of about 20:1. As standard procedure, the packed column was washed with two column volumes of hexane prior to sample loading.

The sample from the saponification procedure which had been reduced to a small volume (a few ml) using an all glass flash evaporator was transferred to the top of the column using a transfer pipette. The saturated hydrocarbons eluted with two column volumes of hexane were set aside for gas chromatography (GLC) and gas chromatography-mass spectrometry (GC/MS). Hexane insoluble material not previously added to the column was washed onto the column with a few milliliters of benzene. The nonsaturated, so-called "aromatic", fraction was eluted with two column volumes of benzene.

The benzene in this fraction was gradually replaced by adding hexane and by evaporating under a stream of nitrogen, taking care not to let the sample go dry at any time, yielding a final volume of 1 ml or less. The hexane eluate was reduced in volume in the same way. The weight, when enough material was present to weigh, of each fraction was determined of an aliquot of the eluates. The samples were set aside and later submitted to GLC and, if appropriate, to GC/MS.

The GLC and GC/MS techniques used are described later in this section of the report.

A tissue sample from the 1976 study, spiked with n-C_{32} and phenanthrene, was taken through the procedures described above and gave 95 and 98% recovery, respectively.

Water-Particulate HMWH

The frozen filters containing particulate hydrocarbons were thawed, placed in a 50 ml flask and extracted with 15 ml hexane on a hot plate at 50°C for at least three hours. The hexane was decanted, replaced with an equal volume of chloroform and the extraction was repeated for an additional three hours at 50°C. The extracts were combined and reduced to near dryness under a nitrogen stream. A small amount of hexane was added continuously to replace the chloroform phase. The hexane was evaporated to about 0.1 ml under a stream of purified nitrogen at room temperature. The sample was transferred to a micro silica-gel-alumina column (0.4 cm x 8 cm). Another 0.4 ml portion of hexane was used to rinse the vial in which the sample was evaporated and this hexane was added to the column. Hexane was used to elute a 0.2 ml initial fraction which was discarded, following which a 2 ml hexane fraction was collected. The non-saturates were eluted with 2 ml of benzene. Hexane and benzene eluates were evaporated under nitrogen to a volume of about 100 μℓ. The samples were tightly sealed in a vial with Teflon-lined caps and further concentrated to a volume of about 25 μℓ (exact volume was measured with a 50 μℓ syringe) just prior to gas chromatographic analysis.

Water-Dissolved HMWH

Samples were extracted with chloroform in a continuous flow extractor as shown in Figure 4.2. The 38-ℓ sample was passed through the apparatus at a flow rate of about 26 ml/minute (38 ℓ in 24 hours). The extraction efficiency of the method was tested during the 1976 STOCS study (Winters, *et al.*, 1976). The results of these tests (Table 4.5) indicated an efficiency of greater than 95% when the flow rate was less than 38 ℓ/18 h. After the water had been extracted, chloroform from the extraction chambers was transferred through glass tubing to a flask in which the pressure had been reduced by means of a small diaphragm pump. Chloroform from the carboys was poured into the same flask. The sides of the extraction chambers and the collection carboys were rinsed with fresh cloroform. The chloroform extracts were then combined and reduced to a volume of 5 ml by dis-

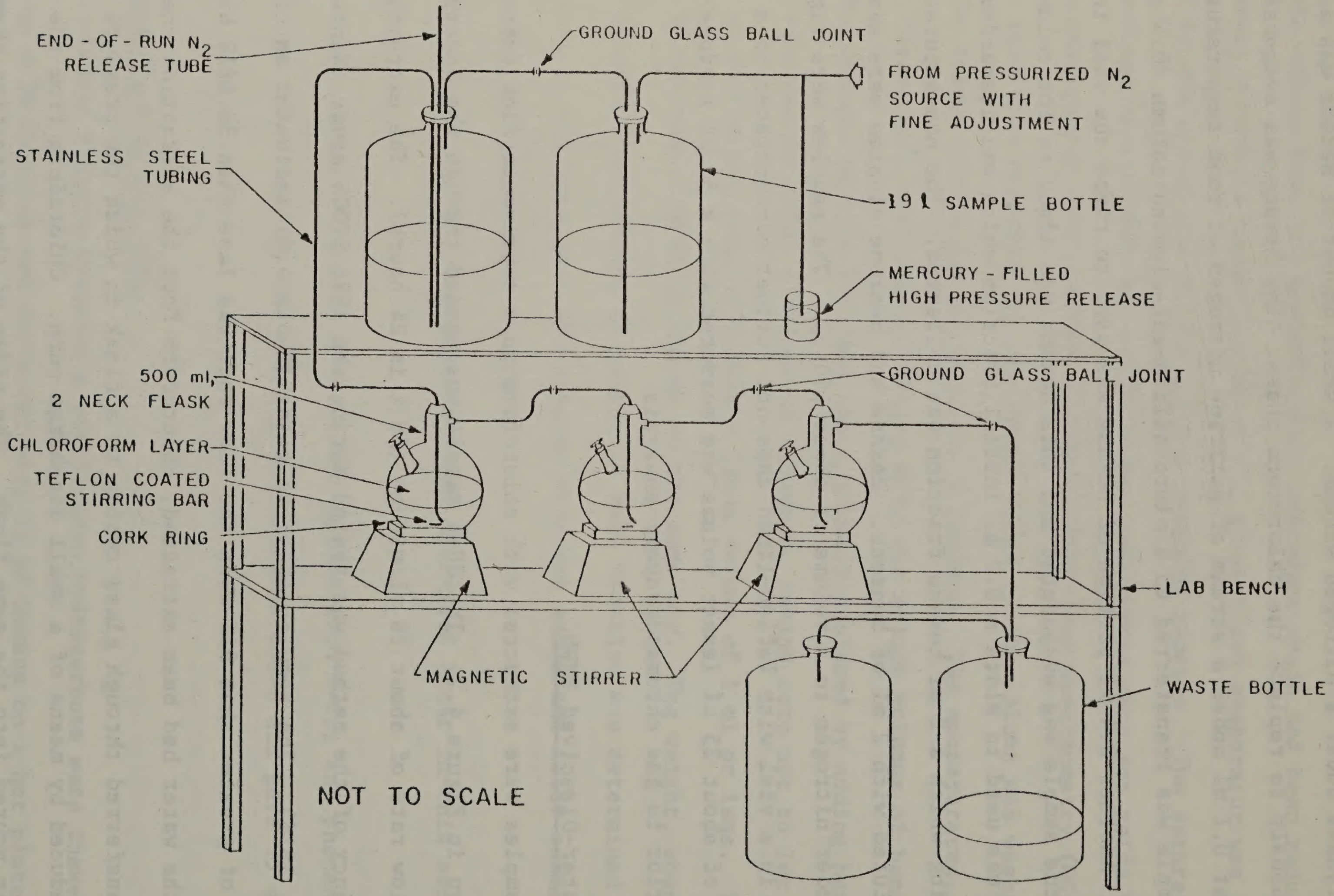

Figure 4.2 Apparatus for the Extraction of Seawater With Chloroform.

TABLE 4.5

EXTRACTION EFFICIENCY TEST RECOVERY OF $n\text{-}C_{21}$ PARAFFIN
ADDED TO 38-l OF FILTERED, EXTRACTED SEAWATER.
CONCENTRATION: 0.13 μg/l

	#1	#2	#3	Av.	Percent Recovery
Standard	1742[1]	1747	1700	1730	100%
Standard Diluted, Evaporated	1727	1837	1682	1749	101%
Test 5 24 hr Extraction	1701	1639	1661	1667	96%
Test 6 24 hr Extraction	1779	1757	1638	1725	100%
Test 7 <18 hr Extraction	1649	1812 (1811)	1648	1702	98%
Test 8 <15 hr Extraction	1487	1506	1546	1513	87%

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[1] Numbers represent electronic integration values

tilling off the chloroform at reduced pressure through a Kuderna-Danish column. The sample was then further concentrated under nitrogen and transferred to a micro silica-gel-alumina column as described for the particulate hydrocarbon samples. The final hexane and benzene eluates were held for GLC and GC/MS analysis.

Sediment

The sediment samples were freeze-dried as the first step of analysis. This was accomplished by spreading the sample as a thin paste on stainless steel trays. The freeze drier used was a VIRTIS Model 25 SRC. The freeze-dried mud was kept frozen until used for analysis.

Hydrocarbons were isoalted from sediments and purified using the reflux method which was used in the 1976 STOCS study. This procedure is as follows: The freeze-dried sediment sample (300-700 g) was placed in a large round-bottomed flask (1-ℓ) and covered (approximately 200 ml) with the toluene-methanol azeotrope (3:7) using care that the flask was not more than one-half full so as to avoid severe bumping on the steambath. The flask was placed in reflux on a steam bath for 14 h using a Friedrichs' condenser with standard taper joints and a drip tip. The solvent, while warm, was decanted through a pre-washed filter (Whatman 541) and set aside for later analysis. Fresh solvent and any sediment on the filter paper were added to the flask and the reflux extraction repeated for 7-10 h. Finally, the sediment was filtered onto a Buchner funnel, the sediment washed with warm hexane and all extracts combined. The sediment was dried at 45°C and weighed. This procedure was repeated several times for each sample in order to process 10 kg.

The combined extracts were taken to just dryness on a roto-vap and taken up in hot KOH-methanol (0.5 N) for saponification. Saponification was carried out according to the procedure described above for zooplankton. No severe problem was encountered with the formation of methyl esters, but in cases where GLC or GC/MS indicated ester formation, the samples were resaponified. Texas coastal sediment is not high in organic matter relative to California basin sediments; for this reason elemental sulfur was not indicated to be a problem in this study. The non-saponifiable fraction obtained in the hexane extract of the saponification mixture was taken to dryness and weighed, yielding the weight of non-saponifiable lipids. The saponifiable fraction was reserved for later fatty acid analyses. The non-saponifiable lipids were submitted to silica-gel-alumina column chromatography according to the scheme described for zooplankton.

The two hydrocarbon fractions isolated from sediments and purified, saturated and non-saturated, were finally taken up in a small volume of hexane (0.05 to 0.5 ml) for GLC and GC/MS analysis.

HMW Hydrocarbon Analyses in Support of Microbiology Projects

A large suite of samples (324) were processed for HMWH in support of the water column bacteriological and water column and benthic mycology projects. One goal of these projects was to measure the microbial decomposition of a Gulf coast crude oil. The samples supplied to this laboratory were part of that research. The results and discussion of the microbiology are reported in Chapters 8 and 9 of this report.

All samples were received frozen or as benzene extracts. The samples were oil-microbe-water mixtures which had been incubated to test for petroleum decomposition. The same crude oil from South Louisiana was used in all experiments. Water Column samples were thawed and extracted twice with 20 ml of benzene. Benthic samples were extracted in a soxhlet extractor with 100 ml of methanol, followed by 100 ml of benzene. All extracts were taken to near dryness and by repeated addition of heptane converted to a heptane solution. The heptane solution was submitted to silica gel fractionation using the method described earlier and the saturated and aromatic fractions collected. The two fractions were taken to a 1 ml volume and GLC analyses carried out using the standard methods. Since these oils are fairly well characterized it was possible to report n-alkanes, pristane, phytane, and seven other branched compounds. The aromatic fraction was analyzed for 17 selected peaks. The data are reported in the above mentioned chapters.

Instrumentation

Gas Chromatographic Analyses

The primary tool for component identification and quantification used in this project was the gas chromatograph (GLC). Identification by GLC is accomplished by comparison of the relative retention times of the unknown compounds with those of selected known standard compounds. Such identification techniques are reasonably valid if the mixture is not complex and expected components are encountered.

The GLC instruments used in this study were Perkin-Elmer[1] (PE) Models 900, 910, 3920B, and a Varian Model 3700. The PE 910 had glass capillary columns. All instruments were equipped with dual column flame ionization detectors. Electronic integration of peaks was done for all instruments by a Hewlett-Packard[1] 3352 Lab Data System. However, due to the complex nature of the GLC patterns and the limitations, of all the integrators, it was necessary to hand check each GLC.

GLC instruments, other than the PE 910, used 0.32 cm (1/8 in.) x 183 cm

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[1] Use of brand names does not constitute an endorsement but is included for descriptive purposes only.

(6 ft) dual, packed columns to effect the separation. The column packing material was 60-80 mesh Gaschrom Q (acid-washed) with a five percent by weight loading of FFAP (product of Varian Corp.) as the stationary liquid-phase. Generally, the operating conditions were as given in Table 4.6. These columns and conditions were used for virtually all analyses of STOCS samples. At the end of the study time a 20 m glass capillary column coated with SP-1000 or OV101 became operable and a few STOCS samples were run on it.

The high temperature to which these columns were subjected was higher than that recommended by the liquid-phase manufacturer. For this reason, the columns had a large amount of column "bleed" at the high temperature which shortened the useful life. Approximately 100 samples could be analyzed before the resolution was considered too poor to permit further analysis.

Instrument sensitivity and resolution were checked daily by running a standard mixture of components. When the resolution fell below that recommended in Attachment A(Contract AA550-CT7-11), the GLC columns were replaced. The daily standard check was used to establish the sensitivity of the instrumentation to allow quantification of the GLC peak data.

GLC peak data for each sample are presented in Appendix C, Tables 1-4. The data consist of a listing of peak retention indices and concentrations in the sample for each of the two analyzed fractions: hexane eluate and benzene eluate from liquid column chromatography. The retention index used was normalized to the relative retention times of the n-alkanes. Thus, for example, the hydrocarbon n-hexadecane had a relative retention index equal to 1600, n-heptadecane equal to 1700, etc. Hydrocarbons having intermediate retention times between n-alkanes were assigned interpolated retention indices; for example, pristane (19 carbon atoms) had a retention index

TABLE 4.6

OPERATING CONDITIONS FOR GLC ANALYSIS

Carrier Gas	Helium
Carrier flow rate	30 ml/min.
Flame detector gas flow rates	
Hydrogen	30 ml/min.
Air	300 ml/min.
Temperature programming	
Initial temperature	70°C
Initial temperature hold time	6 min.
Program rate of rise	6°C/min.
Final temperature	270°C
Final temperature hold time	24 min.

of 1670 and phytane (20 carbon atoms) a retention index of 1780 in as much as their peaks were eluted prior to elution of n-heptadecane and n-octadecane, respectively, on the columns in this study. Retention indices depend upon the nature and molecular size of the component being eluted. Thus, on FFAP, branched chain hydrocarbons elute earlier than the straight chain homologs of the same molecular weight while unsaturation of carbon to carbon bonds will cause the component to elute later than the saturated compound having the same number of carbon atoms.

Gas Chromatography-Mass Spectrometer-Computer Analyses

Where complex component mixtures were to be analyzed it was necessary to augment the chromatographic technique with other organic compound identification methods. One of the more powerful methods was mass spectrometry. Gas chromatography combined with mass spectrometry GC/MS was applied to many of the samples also characterized by gas chromatography alone. A computerized data system was used to assist with data acquisition and data analysis.

The 1977 contract called for GC/MS analysis of 10% of the sample fractions generated in the study. In all, 60 zooplankton, 56 water filtrates, 56 water particulate and 15 sediment extracts were generated with two fractions for each sample. In addition, 19 sediment samples were processed in conjunction with the Topographic Features Study. Thus, 206 samples or 412 fractions were processed. Because of the technical problems associated with the acquisition and interpretation of a large suite of samples only a limited number of the 41 contracted GC/MS analyses are available for inclusion in this report. The remaining analyses and data will be reported in an addendum to this report.

The instrument used was a DuPont Instruments Model 21-49 GC/MS with a DuPont Instruments Model 21-094B MS Data System. The chromatograph associated with this instrument was a Varian-Aerograph Model 2700 modified by DuPont for this service. The effluent from the single chromatographic column was split 9:1 with the major portion of the sample going to the mass spectrometer and the minor portion to a flame ionization detector.

The chromatographic column and conditions used for GC/MS analysis were identical to those used in standard GLC techniques.

It was recognized that for this column these conditions were not necessarily the best for general GC/MS work and that column "bleed" above 220°C was high for GC/MS analyses. However, these parameters were the same as those used in the standard GLC analyses of the samples and, thus, the interpretation of the data was enhanced by direct comparison of the two data sets.

The mass spectrometer was operated with a source temperature of 200°C, electron accelerating potential of 70 volts and an ion accelerating potential of approximately 1400 volts. The mass range from above m/e = 500 to below m/e = 40 was continuously scanned.

The instrument is capable of unit resolution at m/e $\bar{>}$ 1100 but slits and focussing parameters were adjusted for maximum sensitivity at m/e ~ 600. Sensitivity was estimated at better than 1.5 ng hydrocarbon at molecular weight 282 in the reconstructed chromatogram. Specific ion mass-chromatograms effectively allowed even better sensitivity.

RESULTS AND DISCUSSION

Delta C^{13} and Total Organic Carbon

The results of the Delta C^{13} and total organic carbon analyses are summarized in Table 4.7. There was a very clear trend of increasing total organic carbon with distance from shore. This trend correlated with the

TABLE 4.7

SUMMARY OF SEDIMENT DELTA C^{13} AND PERCENT TOTAL ORGANIC CARBON DATA

Line I	Nearshore	Mid Shelf	Offshore	Line Average
Winter	-19.92(.72)	-20.40(.88)	-20.24(1.02)	-20.18(.87)
Spring	-19.58(.47)	-20.50(1.06)	-20.46(1.04)	-20.18(.86)
Fall	-19.24(.58)	-19.68(.94)	-19.89(.56)	-19.60(.69)
Yearly	-19.58(.58)	-20.20(.96)	-20.20(.88)	-19.99(.81)
Line II				
Winter	-20.35(.70)	-20.35(.88)	-20.50(1.12)	-20.40(.90)
Spring	-20.17(.93)	-20.38(.89)	-20.36(1.13)	-20.30(.98)
Fall	-19.43(.82)	-19.65(1.02)	-20.24(1.28)	-19.77(1.04)
Yearly	-19.98(.82)	-20.12(.93)	-20.36(1.18)	-20.17(.97)
Line III				
Winter	-19.75(.94)	-19.90(1.02)	-20.10(.84)	-19.92(.94)
Spring	-19.54(.44)	-19.95(.97)	-20.32(1.12)	-19.94(.34)
Fall	-18.94(.42)	-19.98(1.01)	-19.88(1.30)	-19.60(.91)
Yearly	-19.40(.60)	-19.94(1.00)	-20.10(1.08)	-19.82(.90)
Line IV				
Winter	-19.40(.73)	-20.10(.77)	-20.30(1.10)	-19.99(.90)
Spring	-19.18(.28)	-19.75(.79)	-19.91(.79)	-19.61(.62)
Fall	-19.32(.21)	-19.99(1.75)	-20.26(.86)	-19.86(.94)
Yearly	-19.30(.50)	-19.94(.82)	-20.16(.52)	-19.82(.82)

	Bank 8	Bank 9	Bank Average
Winter	-20.35(1.01)	-20.30(.70)	-20.32(.86)
Spring	-20.26(1.04)	-20.38(1.12)	-20.32(1.08)
Fall	-20.32(1.03)	-20.19(1.22)	-20.26(1.12)
Yearly	-20.31(1.03)	-20.29(1.04)	-20.30(1.04)

	Line I	Line II	Line III	Line IV
Nearshore	4,1	1,4	4,1	4,1
Mid Shelf	2,5	2,5	5,2	5,2
Offshore	6,3	6,3	3,6	6,3,7

percent clay in the samples. There was a less well defined but significant change in Delta C^{13} with slightly more positive (^{13}C enriched) values nearer shore. Seagrasses are more ^{13}C enriched than plankton and this trend may represent the export of seagrasses from the estuary to the shelf especially along Transects I and II. The bank stations were very uniform.

The rather uniform pattern of Delta C^{13} and the low values of total organic carbon suggest that petroleum pollution at a fairly gross level could be detected by Delta C^{13} shifts. If oil of Delta C^{13} equal to -30 is added to sediment at a level to shift the total organic carbon level from 0.5 to 1.0, then the Delta C^{13} will shift from -20 to -25. Such a total organic carbon shift could go undetected but such a Delta C^{13} shift would be easily noted. Even if the oil lost its chemical identity as a hydrocarbon, due to partial oxidation and incorporation into cells, the Delta C^{13} shift would persist.

Zooplankton

The data from GLC analysis of 60 zooplankton samples are presented in Table 1, Appendix C. Illustrations of the n-alkanes distributions and Odd-Even Preference (OEP) curves are given in Figure 1, Appendix C.

Inspection of these figures and tables for smooth n-alkanes distributions and OEP values near unity indicate that about one-half (28/60) of the samples showed the possible presence of petroleum-like organic matter. This was slightly more than was observed in samples collected in 1976 (30%) and considerably higher than observed in 1975 (7%). This apparent increase may reflect the increased import activities for crude oils in the STOCS area. It is not likely that STOCS exploration activities have grown for a size that pollution effects would be observed.

Tables 4.8, 4.9 and 4.10 list various "ratio" type parameters for these zooplankton analyses. Mean values for these parameters are presented in Table 4.11. Correlations which were apparent among these averaged data were seemingly without significance. For instance, the correlation of OEP with season which appeared to be very strong was without significance since a "t" test of the data used to obtain the means shows that there was no significant difference between the greatest value (1.30 for winter) and the least value (1.15 for fall).

Similar trends were tested and found lacking in significance. For example, pristane/phytane ratio vs. season, pristane/phytane ratio vs. OEP, pristane/phytane ratio vs. transect (data not shown), were possible correlations which failed to pass statistical tests of significance.

The criteria for presence of petroleum-like organic matter are smooth distributions of n-alkanes in the region of molecular size greater than C_{21} and OEP values close to unity. In the case of samples analyzed by GC/MS techniques, the presence of aromatic compounds is usually indicative of petroleum-like material.

Table 4.12 lists seven zooplankton samples for which the benzene eluate portion was investigated by GC/MS analysis. One sample, 3/IV, Spring contained polynuclear aromatic hydrocarbons (PAH) in quantities such that they were readily identified even though the quantities were too inadequate for the components to be observable in the gas chromatographic analysis. Four samples, (1/II, Spring; 2/III, Spring; 3/II, Spring; 1/IV, Spring), showed possible trace quantities of PAH's by GC/MS analysis though quantities were inadequate to permit certain identification. Two samples, (2/IV Winter; 1/III, Fall) showed no indication of presence of PAH's. All seven of these samples met the n-alkanes distribution criterion as possibly having petroleum-like organic matter present.

TABLE 4.8

HEAVY HYDROCARBON ANALYSES - STOCS - 1977

SAMPLE TYPE : ZCT

PERIOD : WINTER

CODE	LOCAT.	PR / PH	PR/C-17	PH/C-18	OEP
BABL	1 / I		.54		
BABJ	1 / I				
BABP	1 / I		.46		
BADG	2 / I	33.51	4.98	.21	.48
BAFH	3 / I	117.54	14.46	.22	1.29
BAHV	1 / II	148.12	6.26	.79	1.33
BAIR	1 / II	45.00	5.70	.23	1.22
BAIC	1 / II		5.87		
BAJX	2 / II		11.85		
BAMF	3 / II		19.96		1.60
BARL	1 / III	38.98	.92	.21	
BARQ	1 / III	161.33	1.05	.07	
BARP	1 / III	142.57	.94	.07	
BATG	2 / III	49.97	9.66	.25	
BAVG	3 / III		3.95		2.51
BAXE	1 / IV	200.28	3.96	1.81	1.02
BAZJ	2 / III	57.86	15.61	.40	2.23
BAXK	2 / III	461.07	14.08	.14	
BAZD	2 / IV	38.80	6.63	1.54	.99
BBBD	3 / IV	266.62	7.00	.24	.35

TABLE 4.9

HEAVY HYDROCARBON ANALYSES - STOCS - 1977

SAMPLE TYPE : ZCT PERIOD : SPRING

CODE	LOCAT.	PP / PH	PR/C-17	PH/C-18	DEP
8IYD	1 / I				1.09
8JAG	2 / I				1.13
8JAV	2 / I	346.93	13.94	.14	1.29
8JAA	2 / I	142.69	4.16	.41	1.17
8JEK	1 / II	22.22	4.27	.60	1.18
8JHA	2 / II		10.67		1.07
8IHG	2 / II	171.16	9.25	.36	1.49
8JHI	2 / II	99.61	5.92	.54	1.18
8IIF	3 / II	644.52	6.14	.06	1.41
8JME	1 / III	194.44	8.23	.22	1.17
8JMF	2 / III	37.09	3.62	.57	1.10
8JOK	3 / II	66.72	4.74	.31	1.15
8JOL	3 / II	14.78	3.01	.56	1.09
8JQH	3 / III	190.39	1.71	.12	1.54
8JST	1 / IV	295.42	5.23	.10	.98
8JUJ	2 / IV	583.75	5.62	.12	1.57
8JUU	2 / IV	3.83	4.57	13.97	1.55
8JUP	2 / IV		5.15		
8JML	3 / IV	13.21	2.22	.14	1.22
8KCT	3 / I	25.27	1.31	.54	1.16

TABLE 4.10

HEAVY HYDROCARBON ANALYSES - STOCS - 1977

SAMPLE TYPE : ZCT

PERIOD : FALL

CODE	LOCAT.	PR / PH	PR/C-17	PH/C-18	OEP
80HX	1 / I	164.91	1.25	.14	1.09
80CC	1 / I	37.74	1.64	.32	1.18
80CD	1 / I		.32		.79
80DX	2 / I		8.15		
80FZ	3 / I	43.53	1.25	.11	1.33
80IO	1 / II	2835.48	118.16	.35	.97
80IU	1 / II	282.63	121.03	.42	.91
80IV	1 / II	.27	.43	.54	1.00
80K0	2 / II		7.26		1.51
80MU	3 / II	326.55	2.35	.09	1.22
80P2	1 / III	237.06	8.27	.11	1.28
80PV	1 / III	859.75	7.01	.11	2.26
80P4	1 / III		5.18		.90
8090	2 / III	1373.86	26.93	.04	1.39
80TS	3 / III	39.54	1.50	.42	1.05
80VP	1 / IV	41.08	23.78	.74	1.06
80VU	1 / IV	146.93	20.43	.38	1.12
80VV	1 / IV	1.15	.37	.89	.94
80X2	2 / IV	125.39	55.95	.66	.75
80Z8	3 / IV		.88		

TABLE 4.11

MEAN VALUES FOR RATIO PARAMETERS FOR ZOOPLANKTON SAMPLES

Season	Pristane/ Phytane	Pristane/ nC_{17}	Phytane/ nC_{18}	Odd-Even Preference
Winter	136	7.0	0.4	1.30
Spring	178	5.5	1.2	1.22
Fall	434	20.9	0.3	1.15

TABLE 4.12

ZOOPLANKTON SAMPLES FOR WHICH THE BENZENE FRACTION HAS BEEN INVESTIGATED BY GC/MS ANALYSIS

Sample Code	Station/Transect	Season	Remarks
BAZD	2/IV	Winter	no indication of PAH*
BJEX	1/II	Spring	traces of PAH
BJOF	2/III	Spring	traces of PAH
BJOL	3/II	Spring	traces of PAH
BJSI	1/IV	Spring	traces of PAH
BJWL	3/IV	Spring	good indication of PAH
BOPW	1/III	Fall	no indication of PAH, separation of saturates and unsaturates poor.

* PAH – polynuclear aromatic hydrocarbons

Some of the major peaks in the gas chromatographs of the benzene fraction have been identified by GC/MS analysis. Table 4.13 lists the average retention index and the probable compound identification for some of the more frequently encountered compounds. The computer program used to group the data for generation of this table had more stringent "clustering" requirements than that used in past reports. The net result was that the "average" retention index reported for a component was somewhat more precise and was generally 30 to 60 index units heavier than previously reported values.

Water

Particulate-Hexane Eluate

Total n-alkanes for each sample of particulate organics analyzed were calculated from data included in Table 2, Appendix C, and is presented in Figure 4.3. The concentration of n-alkanes ranged from 0.003 to 1.60 μg/ℓ. About 90% ($^{31}/_{35}$) of the values fell between 0.01 and 0.50 μg/ℓ. These concentrations were similar to those reported during 1976.

The data indicated a trend toward higher concentrations at the inshore stations as was observed in both the 1975 and 1976 STOCS studies. In 6 of the 12 transects (4 transects x 3 seasons) in Figure 4.3, Stations 1 had a higher concentration than Stations 2 or 3. Stations 2 had the highest concentration in 3 of the 12 transects.

The 1977 seasonal averages of all stations were calculated to be: 0.22 μg/ℓ for winter; 0.25 μg/ℓ for spring; and 0.09 μg/ℓ for fall. Seasonal values for 1976 were: winter, 0.31 μg/ℓ; spring, 0.12 μg/ℓ; and fall 0.31 μg/ℓ. Calder (1977) reported the following seasonal values for the MAFLA sutdy area: winter, 0.31 μg/ℓ; summer, 0.14 μg/ℓ; and fall, 0.06 μg/ℓ.

Percentage composition of the n-alkanes in particulate matter did not

TABLE 4.13

SOME GAS CHROMATOGRAPHIC PEAKS OF SIGNIFICANCE BENZENE ELUATES OF ZOOPLANKTON EXTRACTS

Retention Index	Formula	Identification by GC/MS
1910	$C_{14} H_{26}$	14:2 hydrocarbon
2131	$C_{16} H_{30}$	16:2 hydrocarbon (probably cyclohexylclecne
2204	$C_{21}H_{38}$	21:3 hydrocarbon (probably branche chain)
2222	$C_{15}H_{31}COOOCH_3$	Methyl ester of C_{16}:0 fatty acid
2340	$C_{18}H_{34}$ and $C_{20}H_{30}$	18:2 hydrocarbon and 20:6 hydrocarbon
2472	$C_{25}H_{46}$	25:3 hydrocarbon
2649	?	Phthalate ester
3366	?	Phthalate ester

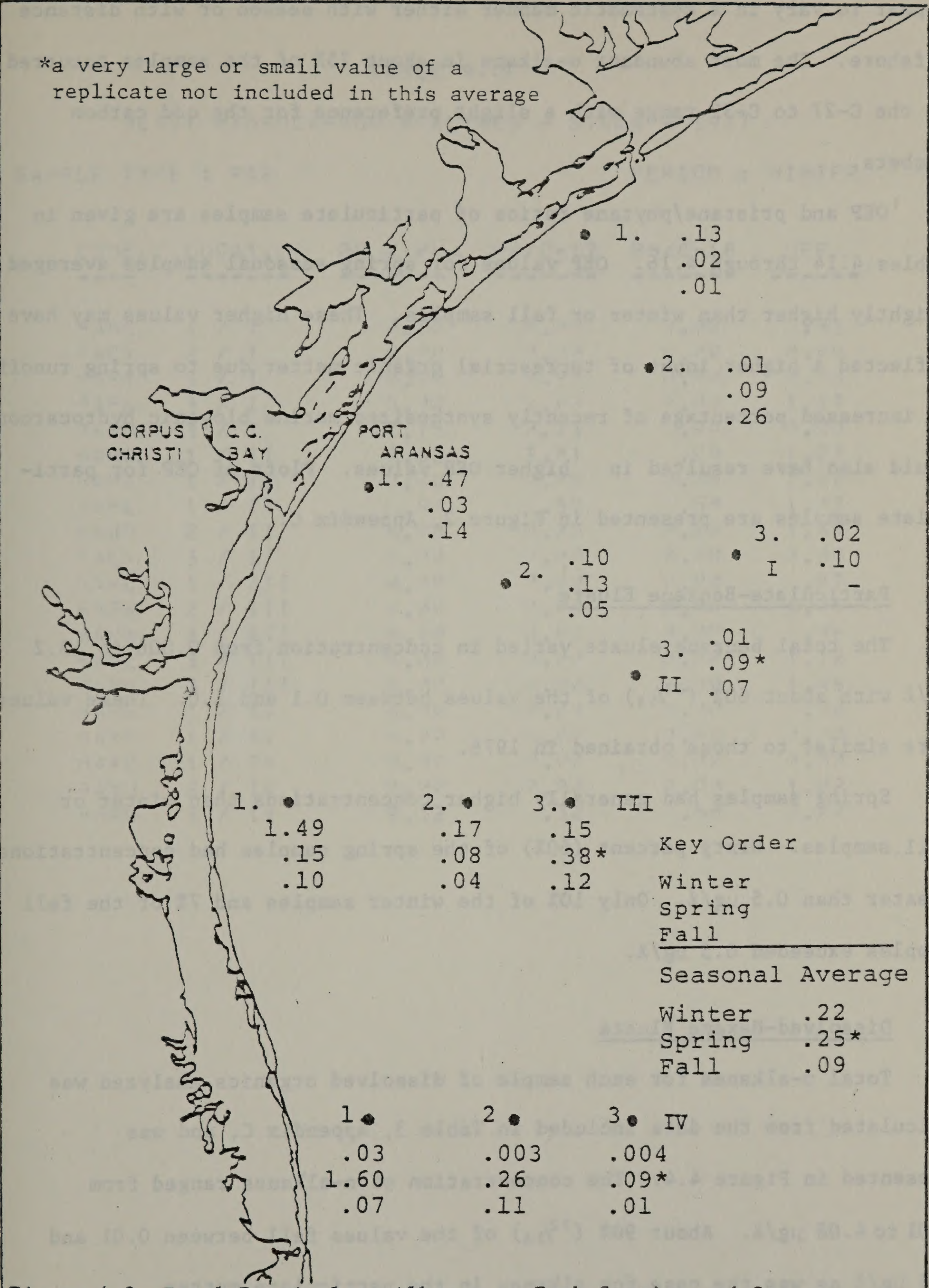


Figure 4.3 Total Particulate Alkanes at Each Station and Season, µg/ℓ.

appear to vary in a systematic manner either with season or with distance offshore. The most abundant n-alkane in about 75% of the samples occurred in the C-27 to C-32 range with a slight preference for the odd carbon numbers.

OEP and pristane/phytane ratios of particulate samples are given in Tables 4.14 through 4.16. OEP values for spring seasonal samples averaged slightly higher than winter or fall samples. These higher values may have reflected a higher input of terrestrial organic matter due to spring runoff. An increased percentage of recently synthesized marine biogenic hydrocarbons could also have resulted in higher OEP values. Plots of OEP for particulate samples are presented in Figure 2, Appendix C.

Particulate-Benzene Eluate

The total benzene eluate varied in concentration from 0.006 to 13.2 µg/ℓ with about 60% ($^{31}/_{55}$) of the values between 0.1 and 1.0. These values were similar to those obtained in 1976.

Spring samples had generally higher concentrations than winter or fall samples. Sixty percent (60%) of the spring samples had concentrations greater than 0.5 µg/ℓ. Only 10% of the winter samples and 7% of the fall samples exceeded 0.5 µg/ℓ.

Dissolved-Hexane Eluate

Total n-alkanes for each sample of dissolved organics analyzed was calculated from the data included in Table 3, Appendix C, and was presented in Figure 4.4. The concentration of n-alkanes ranged from 0.01 to 4.08 µg/ℓ. About 90% ($^{32}/_{35}$) of the values fell between 0.01 and 0.5 µg/ℓ as was the case for alkanes in the particulate matter.

The trend toward higher concentration at inshore stations, seen with

TABLE 4.14

HEAVY HYDROCARBON ANALYSES - STOCS - 1977

SAMPLE TYPE : PAR PERIOD : WINTER

CODE	LOCAT.	PR / PH	PR/C-17	PH/C-18	OEP
RABC	1 / I	0.00	0.00	0.00	.33
RACX	2 / I	0.00	0.00	0.00	0.00
RAEY	3 / I	0.00	0.00	0.00	0.00
RAFM	3 / I	0.00	0.00	0.00	1.17
RAFO	3 / I	17.75	2.73	.44	0.00
RAHG	1 / II	7.32	3.81	.09	1.27
RAHJ	1 / II	0.00	0.00	0.00	1.00
RAHL	1 / II	.90	.53	.58	1.33
RAJO	2 / II	0.00	0.00	0.00	1.39
RALV	3 / II	0.00	0.00	0.00	0.00
RARC	1 / III	0.00	0.00	0.00	.87
RASX	2 / III	0.00	0.00	0.00	1.26
RAUX	3 / III	0.00	0.00	0.00	.96
RAVL	3 / III	0.00	0.00	0.00	1.12
RAVN	3 / III	0.00	0.00	0.00	1.46
RAWV	1 / IV	0.00	0.00	0.00	0.00
RAXM	1 / IV	0.00	0.00	0.00	0.00
RAXO	1 / IV	0.00	0.00	0.00	0.00
RAYU	2 / IV	0.00	0.00	0.00	1.02
RBAU	3 / IV	2.12	.32	.68	0.00

TABLE 4.15

HEAVY HYDROCARBON ANALYSES - STOCS - 1977

SAMPLE TYPE : PAR PERIOD : SPRING

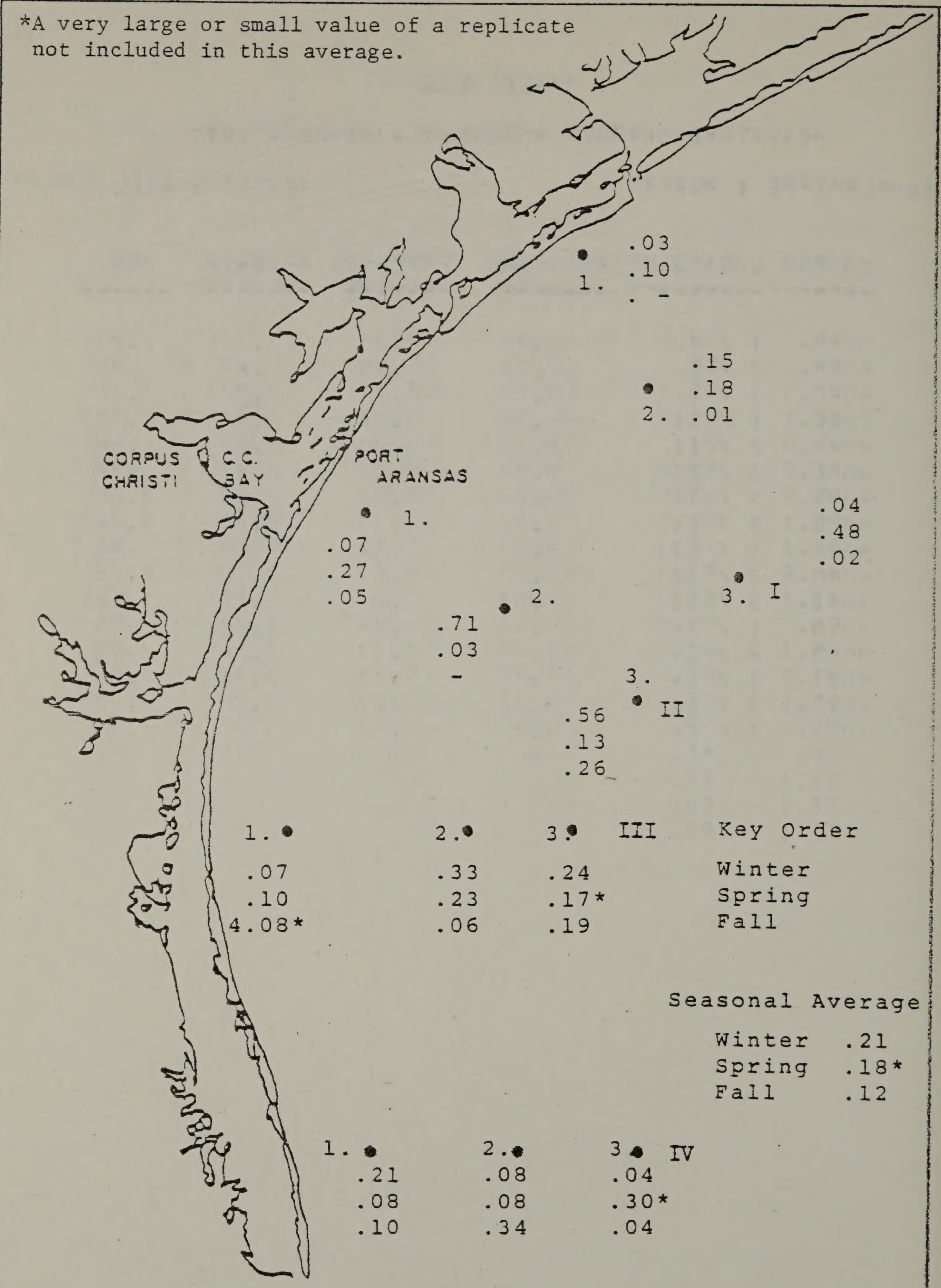
CODE	LOCAT.	PR / PH	PR/C-17	PH/C-18	OEP
8IYF	1 / I	0.00	0.00	0.00	.88
8JAH	2 / I	2.76	.54	.37	.87
8JEN	1 / II	.50	.63	1.30	.54
8JGR	2 / II	0.00	0.00	0.00	1.33
8JIW	3 / II	4.68	.50	.15	0.00
8JJM	3 / II	0.00	0.00	0.00	3.33
8JJO	3 / II	0.00	0.00	0.00	0.00
8JLV	1 / III	.43	.17	1.42	1.29
8JNW	2 / III	0.00	0.00	.51	1.42
8JPY	3 / III	9.97	.43	.07	2.86
8JQN	3 / III	0.00	0.00	.13	1.16
8JQP	3 / III	0.00	.42	0.30	.89
8JSA	1 / IV	.83	.39	.56	1.24
8JUA	2 / IV	.60	.08	.76	2.16
8JWC	3 / IV	2.13	.48	.30	1.70
8JWR	3 / IV	0.00	0.00	.22	0.00
8JWT	3 / IV	1.10	.41	.38	.99
8KCK	3 / I	.47	.02	.72	1.12
8KCZ	3 / I	1.02	.03	.43	1.37
8KDB	3 / I	.73	.10	.89	1.11

TABLE 4.16

HEAVY HYDROCARBON ANALYSES - STOCS - 1977

SAMPLE TYPE : PAR PERIOD : FALL

CODE	LOCAT.	PR / PH	PR/C-17	PH/C-18	OEP
80BD	1 / I	0.00	0.00	0.00	1.03
80DD	2 / I	.75	.35	.99	.91
80FQ	3 / I	0.00	0.00	0.00	0.00
80IE	1 / II	0.00	0.00	0.00	2.07
80KH	2 / II	0.00	0.00	0.00	.64
80ML	3 / II	0.00	0.00	0.00	.97
80NA	3 / II	0.00	0.00	0.00	0.00
80NC	3 / II	0.00	0.00	0.00	1.06
80PH	1 / III	0.00	.22	0.00	.96
80RH	2/ III	0.00	0.00	0.00	2.06
80TJ	3 / III	.43	.14	.75	1.16
80VG	1 / IV	.44	.04	2.08	.99
80XG	2 / IV	0.00	0.00	0.00	0.00
80ZI	3 / IV	0.00	0.00	2.31	0.00
80Z[illegible]	3 / IV	0.00	0.00	0.00	0.00
80ZY	3 / IV	0.00	0.00	0.00	.95



*A very large or small value of a replicate not included in this average.

Figure 4.4 Total Dissolved Alkanes at Each Station and Season, μg/ℓ.

particulate samples and to some extent with dissolved samples in 1976, was not observed in the 1977 data (Figure 4.4).

Seasonal averages of all stations were as follows: winter, 0.21 µg/ℓ; spring, 0.18 µg/ℓ; and fall, 0.12 µg/ℓ. These values were quite similar to seasonal averages last year which were 0.24, 0.14 and 0.11 µg/ℓ, respectively.

Percentage composition of n-alkanes in the dissolved fraction was similar to that found in the particulate samples. The most abundant alkane in more than 70% of the samples contained between 27 and 33 carbons with a strong preference for the odd numbers.

Pristane/phytane ratios and OEP values for dissolved samples are given in Tables 4.17 through 4.19. Winter and spring average OEP values were similar and somewhat higher than the fall average. Plots of OEP for dissolved samples are presented in Figure 3, Appendix C.

Dissolved-Benzene Eluate

The total benzene eluate from dissolved organics varied in concentration from 0.013 to 107.7 µg/ℓ. Of those samples with concentrations greater than 1 µg/ℓ, about 75% had only one or two major components. The major components appeared to be biogenic compounds, such as squalene and other polyolefinic hydrocarbons, and phthalates. No systematic variation in concentration was apparent either with season or distance offshore.

Sediment HMWH

The results of the HMW hydrocarbon analyses of sediment samples are given in Table 4, Appendix C, and include both saturated and non-saturated hydrocarbons. The results were in agreement with the Delta C^{13} and total organic carbon data in that the very low level of saturated hydrocarbons

TABLE 4.17

HEAVY HYDROCARBON ANALYSES - STOCS - 1977

SAMPLE TYPE : DIS PERIOD : WINTER

CODE	LOCAT.	PR / PH	PR/C-17	PH/C-18	OEP
8ABD	1 / I	0.00	0.00	0.00	0.00
8ACY	2 / I	0.00	0.00	0.00	.29
8AEZ	3 / I	0.00	0.00	0.00	1.36
8AFN	3 / I	0.00	0.00	0.00	.98
8AFP	3 / I	0.00	0.00	0.00	.89
8AHI	1 / II	2.50	.29	.13	1.06
8AHK	1 / II	0.00	0.00	0.00	1.01
8AHM	1 / II	0.00	0.00	0.00	1.42
8AJP	2 / II	0.00	0.00	.55	1.10
8ALN	3 / II	1.48	.49	.20	1.52
8ARD	1 / III	0.00	0.00	0.00	1.10
8ASY	2 / III	0.00	0.00	0.00	1.10
8AUY	3 / III	0.00	0.00	0.00	.96
8AVM	3 / III	0.00	0.00	0.00	1.14
8AVO	3 / III	0.00	0.00	0.00	1.65
8AWN	1 / IV	0.00	0.00	0.00	1.12
8AXN	1 / IV	0.00	0.00	0.00	.93
8AXP	1 / IV	0.00	0.00	0.00	0.00
8AYV	2 / IV	0.00	0.00	.44	0.00
8BAV	3 / IV	6.65	0.00	.05	0.00

TABLE 4.18

HEAVY HYDROCARBON ANALYSES - STOCS - 1977

SAMPLE TYPE : DIS PERIOD : SPRING

CODE	LOCAT.	PR / PH	PR/C-17	PH/C-18	OEP
8IYG	1 / I	0.00	0.00	1.93	.84
8JAI	2 / I	1.25	.49	.54	1.04
8JEO	1 / II	1.91	.54	.31	.50
8JGS	2 / II	0.00	0.00	0.00	1.28
8JIX	3 / II	1.82	.54	.35	1.08
8JJN	3 / II	.83	.98	3.64	1.27
8JJP	3 / II	0.00	0.00	0.00	1.16
8JL[illegible]	1 / III	.13	.49	3.84	.95
8JNX	2 / III	6.16	.42	.10	1.03
8JPZ	3 / III	.51	.73	.35	1.03
8JQO	3 / III	6.00	.35	.08	1.06
8JQQ	3 / III	0.00	.33	0.00	1.04
8JSB	1 / IV	0.00	0.00	1.24	1.41
8JUR	2 / IV	1.54	.53	.38	1.43
8JWD	3 / IV	4.54	.48	.20	1.04
8JWS	3 / IV	0.00	0.00	0.00	0.00
8JWU	3 / IV	1.49	.49	.37	1.17
8KCL	3 / I	.31	.51	1.39	1.04
8KDA	3 / I	0.00	.27	0.00	1.12
8KDC	3 / I	.79	.31	1.02	1.39

TABLE 4.19

HEAVY HYDROCARBON ANALYSES - STOCS - 1977

SAMPLE TYPE : DIS PERIOD : FALL

CODE	LOCAT.	PR / PH	PR/C-17	PH/C-18	OEP
80BP	1 / I	0.00	0.00	0.00	0.00
80DP	2 / I	0.00	0.00	0.00	0.00
80FR	3 / I	0.00	0.00	0.00	0.00
80IF	1 / II	2.35	4.11	1.12	.98
80KI	2 / II	0.00	0.00	0.00	0.00
80MA	3 / II	.49	4.18	1.33	1.72
80MR	3 / II	.62	2.05	4.93	0.00
80ND	3 / II	0.00	0.00	0.00	.83
80PI	1 / III	.01	.06	1.13	.82
80RI	2 / III	0.00	11.63	0.00	1.15
80TK	3 / III	0.00	0.00	.69	.99
80VH	1 / IV	.66	.51	1.68	1.20
80XH	2 / IV	0.00	0.00	0.00	.71
80ZJ	3 / IV	0.00	0.00	0.00	0.00
80ZX	3 / IV	.41	1.23	.99	0.00
80ZZ	3 / IV	0.00	0.07	0.00	0.00

detected (0.1 to 1.9 µg/ℓ) pointed to a natural biogenic population of hydrocarbon molecules. Data reduction given in Tables 4.20 through 4.22 and Figure 4, Appendix C, support this conclusion. For example, no OEP values near unity were present nor were high pristane to C-17 values reported. With respect to sediment high-molecular-weight hydrocarbons, the STOCS must be viewed as a very clean area. Detailed analyses of the three years of HMWH data as related to other environmental parameters will be carried out in the three year data synthesis.

TABLE 4.20

HEAVY HYDROCARBON ANALYSES - STOCS - 1977

SAMPLE TYPE : CHG PERIOD : WINTER

CODE	LOCAT.	PR / PH	PR/C-17	PH/C-18	OEP
BBED	1 / I	4.41	.62	.21	1.71
BBGC	2 / I	3.13	.32	.17	4.43
BBTA	3 / I	1.86	.39	.33	2.63
BBTZ	1 / IV	3.42	.40	.09	3.64

TABLE 4.21

HEAVY HYDROCARBON ANALYSES - STOCS - 1977

SAMPLE TYPE : CHG PERIOD : SPRING

CODE	LOCAT.	PR / PH	PR/C-17	PH/C-18	OEP
BHHT	1 / II	5.26	.75	.20	4.10
BHKK	2 / II	6.46	1.01	.15	2.08
BHNR	3 / II	1.91	.67	.39	3.72
BIJT	2 / IV	5.73	.88	.31	4.01
BJXT	1 / II	1.89	.54	.43	1.82

TABLE 4.22

HEAVY HYDROCARBON ANALYSES - STOCS - 1977

SAMPLE TYPE : CHG PERIOD : FALL

CODE	LOCAT.	PR / PH	PR/C-17	PH/C-18	OEP
BQGO	2 / III	4.20	.41	.10	3.39
BQTX	3 / IV	4.89	.64	.27	2.95
BSEJ	1 / III	2.06	.45	.46	4.36
BSEK	2 / III	2.95	.51	.20	4.27
BSEL	3 / III	6.65	.46	.13	3.26
BSEM	3 / IV	2.47	.68	.43	2.97

LITERATURE CITED

Calder, J. A. 1977. Seasonal variation of hydrocarbons in the water column of the MAFLA lease area. pp 432-441. *In* Fate and effects of petroleum hydrocarbon in marine organisms and ecosystems. D. A. Wolfe (ed.) Proc. of Symp., November, 1976, Seattle, Washington. Permagon Press.

________. 1969. Carbon isotope effects in biochemical and geochemical systems. Dissertation Ph.D., the University of Texas, Austin, Texas.

Fredericks, A. D., and W. M. Sackett. 1970. Organic carbon in the Gulf of Mexico. J. of Geophysical Res., Oceans and Atmospheres, 75(12): 2199-2206.

Hedges, J. I., and P. L. Parker. 1976. Land-derived organic matter in surface sediments from the Gulf of Mexico. Geo. et Cos Acta, 40(9): 1019-1029.

Mauer, L. G., and P. L. Parker. 1972. The distribution of dissolved organic matter in the nearshore waters of the Texas coast. Contr. in Mar. Sci. 16.

Parker, P. L. 1969. Fatty acids and alcohols. *In* Organic Geochemistry. Methods and Results. Springer-Verlag, New York.

________. 1967. Fatty acids in recent sediments. Contr. in Mar. Sci. 12:135.

Sever, J., and P. L. Parker. 1969. Fatty acids (normal and isoprenoid) in sediments. Sci. 164:1052.

Winters, K., R. O'Donnell, J. C. Batterton, and C. Van Baalen. 1976. Water soluble components of four fuel oils: chemical characterization and effects on growth of microalgae. Mar. Biol. 36:269-276.

CHAPTER FIVE

HEAVY MOLECULAR WEIGHT HYDROCARBONS IN MACROEPIFAUNA AND MACRONEKTON SAMPLES

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ABSTRACT

A total of 207 samples of benthic macroepifauna and macronekton from the South Texas Outer Continental Shelf (STOCS) was analyzed for heavy-molecular-weight hydrocarbons. These analyses included samples of muscle, liver, and gonads. Five (5) large samples, 36 intraspecific variability samples and 3 ship's contaminant samples were also analyzed. The samples generally had very low levels of total hydrocarbons, averaging 2 ppm or less, with C_{15} and C_{17} alkanes and pristane predominating. The aliphatic hydrocarbon distribution and the very low levels of phytane and aromatic hydrocarbons (generally 0.01 ppm or less) detected were suggestive of biogenic origins for the hydrocarbons in the samples analyzed. The absence of correlations in the pristane/phytane, pristane/C_{17}, phytane/C_{18} and CPI_{14-20} ratios also implied the absence of significant levels of petroleum in the organisms analyzed.

INTRODUCTION

Heavy molecular weight hydrocarbons were analyzed in macroepifauna and macronekton samples from the South Texas Outer Continental Shelf (STOCS) in order to obtain hydrocarbon data for these organisms which would characterize the natural quantities observed. There is relatively little information available on the hydrocarbon composition of marine organisms. Thus, the hydrocarbon data obtained from the relatively pristine STOCS provides a needed data base for evaluating the effects of petroleum exploration and production on the hydrocarbon composition of organisms in this area.

The macroepifauna samples analyzed in this study were obtained from the STOCS area during the 1977 study period. The samples represented three classes of organisms (molluscs, crustaceans, and fish) and consisted mainly of fish and shrimp which included all species listed in Appendix D. The macronekton, fish of the snapper family, were obtained from the Southern Bank and Hospital Rock stations during seasonal and monthly cruises.

Laboratory analyses were based on methods outlined in Attachment A of BLM Contract AA550-CT7-11. Our methods are detailed in the following section.

MATERIALS AND METHODS

Background

The sampling scheme and frequency of sampling presented below were followed whenever possible. Epifaunal and demersal fish samples for hydrocarbon analyses were collected three times (seasonally) at Stations 1-3, all transects, with a 35-ft (10.7-m) Texas box otter trawl. The epifaunal samples consisted of five individuals each of three species. In addition, 36 intraspecific variability samples were collected opportunistically to allow the analysis of six individuals of the same species. Five 10-kg samples were also collected opportunistically for intense study of the aromatic fractions. Macronekton were collected at Southern Bank and Hospital Rock seasonally and monthly by hook and line; samples consisted of five individuals of each of two species per bank station. Every reasonable precaution was taken to avoid contamination during sampling. Samples were packed in cleaned glass jars fitted with teflon or aluminum foil. When potentially contaminating sediment or other foreign material was adhering to the organisms they were rinsed with seawater. All samples were immediately frozen and so kept until analysis.

Laboratory Analysis

Materials[1]

Solvents were Mallinckrodt Nanograde^R and were used as received or redistilled when required. Silica gel (Woelm, 70-230 mesh) and Aluminum Oxide Woelm Neutral (Activity Grade 1) were activated at 200°C for at least 24 hr before use. Hydrocarbon standards were obtained from Analabs and Polyscience Co.

Instrumentation

A Hewlett-Packard 5830A gas chromatograph (GC) and a Varian 3700 HC were used. Both were equipped with dual flame ionization detectors, programmable integrators, and 30 to 50 m OV-101 or SE-30 WCOT glass capillary columns. The injector was at 270°C and the detector at 280°C. The column oven was temperature-programmed from 70 to 270°C at 3°/minute.

Procedures

Background Information

Prior to actual sample analysis, procedure blanks and recovery studies were performed. All solvents to be used in the procedure were concentrated to the extent required by the procedure and analyzed by gas chromatography. Any solvent exhibiting any impurities in the hydrocarbon region of the spectrum was rejected or redistilled in an all-glass system. Solid reagents were purified by heating in a 325°C oven for at least 24 h; concentrates of solvent rinses of these materials were inspected by gas chromatography as described for solvents. Glassware and equipment were washed with Micro cleaning solution (International Products Corp.) and distilled water, rinsed with acetone, methanol and hexane and heated overnight at 325°C. After heating, they were rinsed with two portions of benzene and two of hexane. The final hexane rinse was concentrated and checked by gas chromatography. If any impurities were present, rinsing was repeated as needed to obtain an acceptable blank. Glassware checks accompanied each sample run and procedure blanks were performed at frequent intervals[2].

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[1] Trade names of reagents, solvents and equipment, and the suppliers are included to facilitate recognition by interested readers of what we use; there is no implication that these are solely recommended.

Extraction and Saponification of Macrofauna

Approximately 100 g of tissue were used for all analyses, except for the 10-kg samples which were divided into 600-700 g portions for digestion. When possible, a minimum of five organisms or portions thereof was used per analysis to minimize the natural variability of hydrocarbon content in conspecifics. Each sample was macerated and the wet weight determined. An aliquot of the sample was then placed in a tared beaker and dried at 60°C until a constant weight was obtained. In this manner, the wet and dry weights of the sample were obtained. The remainder of the sample was saponified.

Saponification was conducted by refluxing the sample with 0.05 g KOH/g tissue in approximately 50 ml methanol/100 g tissue. Saponification was continued until the tissues were digested. After the completion of digestion, an equal volume of purified water was added to the mixture. The mixture was then refluxed overnight. Upon completion of hydrolysis, the mixture was diluted with an equal volume of a saturated NaCl solution. The mixture was then extracted three times with n-pentane. The volume of n-pentane used for each extraction was equivalent to the volume of methanol initially used in the saponification. The n-pentane fractions were then combined and washed with an equal volume of water. The solvent was removed from the pentane extract (for weight determination) prior to column chromatographic separation.

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[2] Procedure blanks constituted approximately 10% of the total analyses.

Column Chromatography

Weight ratios of about 100 parts alumina to 1 part lipid sample and 200 parts silica gel to 1 part lipid sample were used. The column had a length to inside diameter (i.d.) ratio of approximately 20:1. Both the silica gel and the neutral alumina were Activity I. The column was packed in hexane and rinsed with one column volume of n-pentane. At no time was the column allowed to run dry. The extract taken up in a small volume of n-pentane was then applied to the column and the aliphatic fraction eluted with two column volumes of n-pentane. This was followed by elution of aromatics with two column volumes of benzene. The eluates from the two fractions were then taken to near dryness. They were then transferred to screw cap vials with teflon-lined caps, and the remainder of the solvent was removed with a stream of purified nitrogen. Following column chromatography, all eluates were analyzed by gas chromatography.

Gas Chromatographic Separations

Each eluted fraction obtained from the column chromatographic separation was quantitatively dissolved in a small volume of iso-octane for injection into the GC. A WCOT glass capillary column (30-50 m) of OV-101 or SE-30 was used for the analyses. The column resolved n-C_{17} from pristane and n-C_{18} from phytane with a resolution (R) of approximately unity, where

$R = 2d/w_1 + w_2$ and,

w = the width of each peak at the base on one phase for both pairs of components, and

d = the distance between apices.

The column was also capable of resolution of hydrocarbons from n-C_{14} through n-C_{32}. To assist identification, the following compounds were used as standards to match the retention times of peaks in the gas chromatogram: aliphatic hydrocarbons C_{15}-C_{32}; trimethylbenzene; 1,2,3,5-tetramethylbenzene; 1,2,3,4-tetramethylbenzene; naphthalene; 2-methylnaphthalene; 4-phenyltoluene; 3,3'-dimethylbiphenyl; 4,4'-dimethylbiphenyl; fluorene; 1-methylfluorene; phenanthrene; anthracene; 9-methylanthracene; fluoranthene; and chrysene.

Gas Chromatography-Mass Spectrometry (GS-MS)

Aliquots of extracts from 10% of the GC samples were analyzed by GC-MS. The runs were made by L. Burchfield of the Center for Trace Characterization. Since the concentrations of components were very low (often near the limit of detection of GC-MS), only major compounds found in gas chromatograms were identified.

The analyses were performed with a Hewlett-Packard 5982A dodecapole mass spectrometer interfaced to a 5710A gas chromatograph. This GC-MS system was supported with a 5933A Data System, a Tektronix 4012 CRT terminal, a Tektronix 4631 Hard Copy Unit, and a 15,000 spectra reference library stored on a single disc (Aldermaston).

Capillary columns coated with SE-30 (30 m) or SP-2100 (25 m) were used in the GC. Helium was used as the carrier gas at 36 cm/sec. All samples were run in the splitless mode with injector flush occurring 40 seconds after injection; the injector temperature was 250°C. For the SE-30 column, the temperature program was held at 70°C for four minutes and then raised to 200°C at 2°/minute. For the SP-2100 column, the temperature program was held at 50°C for four minutes and then raised at 4°/minute to 250°C. The column effluent was taken directly into the ion source producing a pressure of 6×10^{-6} torr. The source temperature for all runs was 180°C ± 10°C. The mode of ionization was electron impact using a beam of 70 eV electrons at a current of approximately 200 μa. Mass range was scanned from 50 to 500 amu at a rate of 162 amu/sec.

The total ion chromatogram for each sample was permanently stored on auxillary discs. The spectra were background-subtracted where necessary. Major sample components which appeared in both GC and GC-MS were identified. Some minor sample components were not identified due to poor signal-to background spectra. The electron-impact spectra of individual components were permanently stored on disc for comparison with library spectra or for other uses. Individual spectra from data files were compared as follows: 1) by computer with spectra included in the Aldermaston Library on disc using the "search" routine; 2) with reference spectra run on our instrument; and 3) with the "Eight Peak Index of Mass Spectra" (Mass Spectrometry Data Center). Table 43, Appendix D, shows the compounds that were confirmed by GC-MS for each sample.

Procedure blanks were performed prior to sample analyses and excellent procedure blanks (≤ 0.01 ppm) were routinely obtained. For a detailed discussion of our decontamination procedures, see Giam and Wong (1972) and Giam *et al.* (1975).

TABLE 5.1

PROCEDURAL RECOVERY[1] OF ALIPHATICS AND AROMATICS

COMPOUND	MEAN RECOVERY (%)
n-Pentadecane (C_{15})	78 ± 17
n-Hexadecane (C_{16})	84 ± 12
n-Heptadecane (C_{17})	79 ± 11
Pristane (Pr)	80 ± 11
1-Octadecene ($C_{18:1}$)	69 ± 16
n-Octadecane (C_{18})	79 ± 10
Phytane (Py)	80 ± 12
n-Nonadecane (C_{19})	78 ± 11
1-Eicosene ($C_{20:1}$)	72 ± 13
n-Eicosane (C_{20})	82 ± 11
n-Uncosane (C_{21})	80 ± 11
n-Docosane (C_{22})	81 ± 10
n-Hexacosane (C_{26})	79 ± 20
n-Octacosane (C_{28})	80 ± 13
n-Triacontane (C_{30})	80 ± 17
n-Dotriacontane (C_{32})	92 ± 7
5α-Androstane	82 ± 13
5α-Cholestane	79 ± 14
Naphthalene	51 ± 39
1-Methylnaphthalene	64 ± 33
1,3-Dimethylnaphthalene	80 ± 27
Biphenyl	78 ± 21
Acenaphthene	85 ± 18
Fluorene	81 ± 22
9,10-Dihydrophenanthrene	91 ± 12
Phenanthrene	84 ± 18
3,6-Dimethylphenanthrene	70 ± 13
Pyrene	47 ± 24
Nonadecylbenzene	79 ± 17

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[1] Recovery of hydrocarbons subjected to all steps in the analytical procedure in the absence of biota; 20 analyses were performed.

RESULTS

Twenty recovery studies were carried out on spiked samples and yielded the results in Table 5.1. The limits of detection for our procedure were 0.001 ppm or better. The capillary columns used in these studies gave excellent resolution of the aliphatic hydrocarbons from $\underline{n}$-C_{14} through $\underline{n}$-C_{32}. The chromatogram of the BLM reference mixture (Table 5.2) shown in Figure 5.1 illustrates the resolution obtained (compare to Figure 5.2). Examples of the chromatograms obtained from samples are shown in Figures 1-4, Appendix D.

Analyses of Macroepifauna Samples

The results of the analyses of 109 seasonal macroepifauna samples are detailed in Tables 1-9, Appendix D, as total concentration of alkanes, percent distribution of $\underline{n}$-paraffins, the levels of pristane and phytane, and the ratios of pristane/phytane, pristane/C_{17} and phytane/C_{18} and the carbon preference index (CPI) ratios. In most cases, the C_{15} and C_{17} alkanes were the dominant $\underline{n}$-alkanes. Pristane was present in almost all samples at relatively high levels, while phytane was present in approximately 30% of the samples at concentrations generally less than 0.1 ppm. The pristane/phytane ratios ranged from 2 to 283. The pristane/C_{17} ratios were found to be from 0.3 to 162 while the phytane/C_{18} ratios were mainly in the narrow range from 0.3 to 3. The majority of the CPI_{14-20} ratios were between 0.6 and 31. The CPI_{20-32} ratios were in the range of 0.2 to 20.5 with the majority of values from 0.5 to 3.0. Aromatic compounds were detected in a number of samples, but in quantities too low for quantitation (< 0.005 ppm). GC-MS confirmation was, however, possible for many of the compounds. The compounds detected were 1, 2, 3, 5- and 1, 2, 3, 4-tetramethylbenzene, biphenyl, 2, 6- and 1, 3-dimethylnaphthalene, acenaphthene,

TABLE 5.2

COMPOUNDS AND PEAK REFERENCE NUMBERS OF BLM REFERENCE MIXTURE

1. Naphthalene
2. 1-Methylnaphthalene
3. Biphenyl
4. 1,3-Dimethylnaphthalene
5. Acenaphthene
6. C_{15}
7. Fluorene
8. C_{16}
9. 9,10-Dihydrophenanthrene
10. C_{17}
11. Pristane
12. Phenanthrene
13. $C_{18:1}$
14. C_{18}
15. Phytane
16. C_{19}
17. 3,6-Dimethylphenanthrene
18. 5α-Androstane
19. $C_{20:1}$
20. C_{20}
21. Pyrene
22. C_{21}
23. C_{22}
24. Nonadecylbenzene
25. C_{26}
26. C_{28}
27. 5α-cholestane
28. C_{30}
29. C_{29}

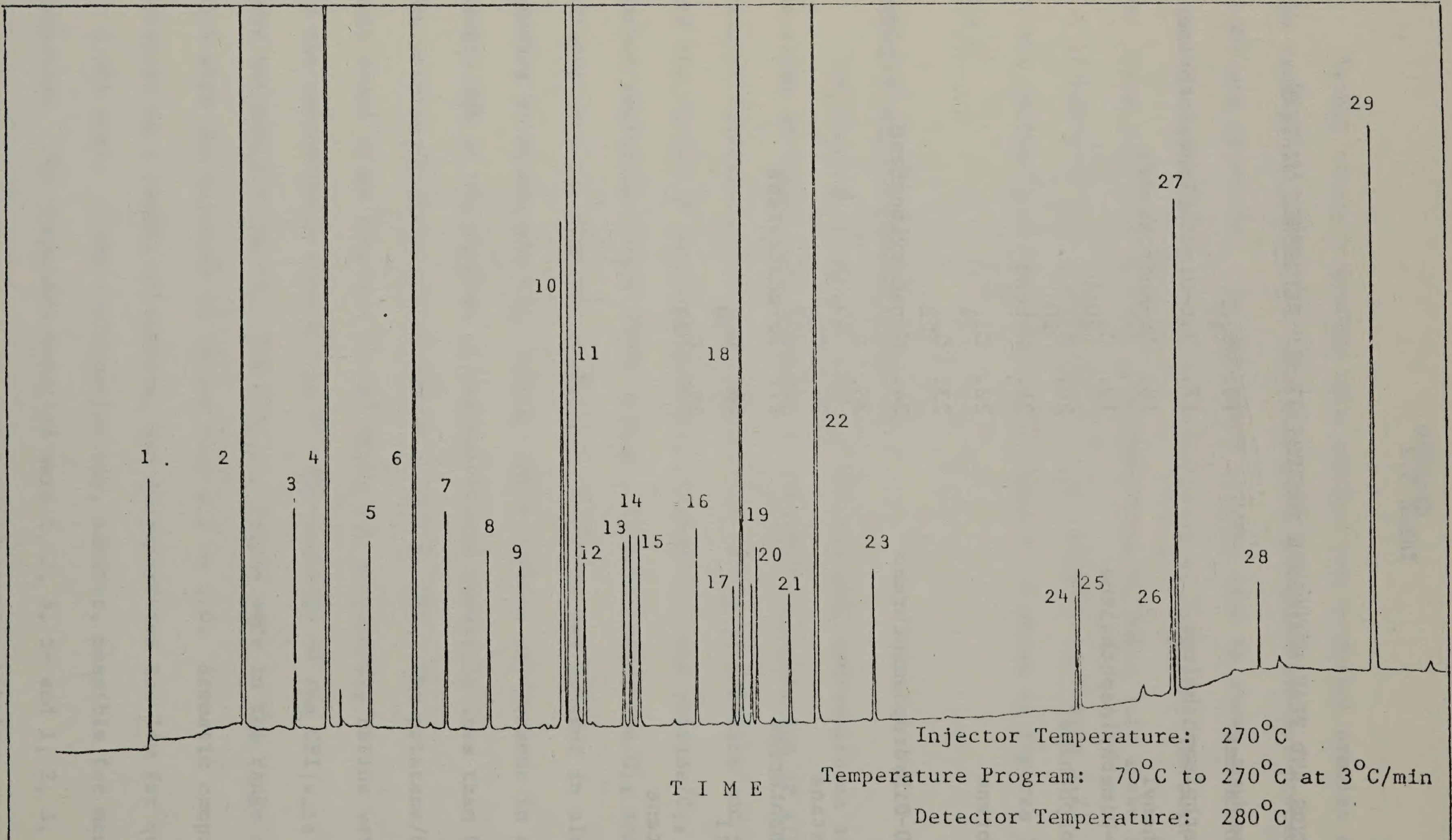

Figure 5.1 Our Gas Chromatogram of BLM Reference Mixture on a 30-m OV-101 WCQT Column. (See Table 2 for compound identification.)

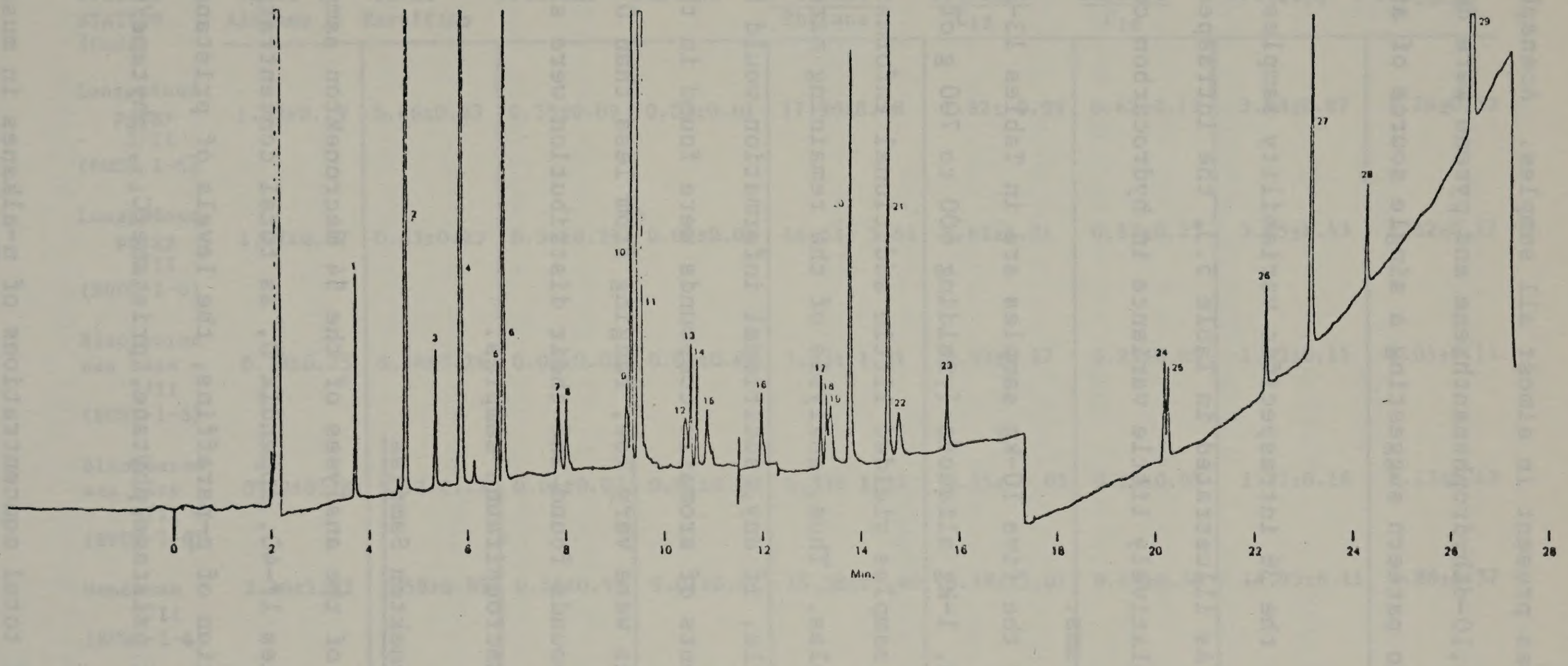

Figure 5.2 Gas Chromatogram Provided by Supelco for the BLM Reference Mixture. (GC Conditions not Supplied; see Table 5.2 for Compound Identification)

4-phenyltoluene, fluorene, 3,3'-dimethylbiphenyl, 9,10-dihydrophenenthrene, 1-methylfluorene, phenanthrene, anthracene, fluoranthene, pyrene, and chrysene. Squalene was present in almost all samples. Acenaphthene, 3,3'-dimethylbiphenyl, 9,10-dihydrophenanthrene and pyrene were detected most frequently, but no pattern suggesting a single source of aromatic compounds was noted.

The data for the 36 intraspecific variability samples is in Tables 10-12, Appendix D. As illustrated in Table 5.3, the intraspecific variability samples showed relatively little variance in hydrocarbon content among individual organisms.

The data for the five 10-kg samples are in Tables 13-16, Appendix D. For these samples, 1-kg aliquots (yielding 600 to 700 g of tissue) were analyzed. These samples yielded little additional information relative to the standard samples. Thus, analysis of the remaining material was not performed as little, if any, additional information would be obtained. Quantifiable amounts of aromatic compounds were found in these samples, but the concentrations were very low, ranging from less than 0.005 to 0.03 ppm. The types of compounds found and their distribution were similar to that found for other macroepifauna samples.

Analyses of Macronekton Samples

The results of the analyses of the 54 macronekton samples are presented in detail in Tables 17-40, Appendix D, as total concentration of alkanes, percent distribution of n-paraffins, the levels of pristane and phytane, and the ratios of pristane/phytane, pristane/C_{17}, phytane/C_{18} and the CPI ratios.

The range of total concentrations of n-alkanes in muscle, liver and gill, as well as the ratios calculated from the data are shown in Table 5.4.

TABLE 5.3

MEAN AND RANGE OF VALUES OBTAINED FROM THE ANALYSIS OF SIX INDIVIDUALS FOR INTRASPECIFIC VARIABILITY

SPECIES STATION (Code)	Total Alkanes	Total Paraffins	Pristane	Phytane	Pristane/Phytane	Pristane/C_{17}	Phytane/C_{18}	CPI_{14-20}	CPI_{20-32}
Longspined porgy 2/II (BUDX-1-6)	1.03±0.19	0.66±0.23	0.35±0.09	0.03±0.01	17.30±8.48	1.82± 0.99	0.62±0.13	3.63±0.87	1.78±0.32
Longspined porgy 3/II (BUDU-1-6)	1.02±0.37	0.63±0.13	0.36±0.29	0.02±0.01	16.63± 5.61	1.62±1.21	0.57±0.22	3.45±1.43	1.52±0.17
Blackeared sea bass 2/II (BUDY-1-6)	0.78±0.25	0.74±0.26	0.03±0.02	0.01±0.00	3.33± 1.51	0.33±0.17	0.25±0.05	1.22±0.15	1.05±0.14
Blackeared sea bass 3/II (BUDV-1-6)	0.77±0.18	0.72±0.17	0.04±0.02	0.01±0.00	4.33± 1.51	0.35± 0.05	0.23±0.05	1.37±0.16	1.13±0.12
Wenchman 2/II (BUDZ-1-6)	2.16±1.22	1.58±0.89	0.56±0.59	0.01±0.02	19.58±22.60	8.78±13.07	0.42±0.58	14.95±6.11	1.88±1.57
Wenchman 3/II (BUDW-1-6)	4.36±4.63	3.40±3.26	0.95±1.54	0.02±0.02	32.42±28.72	0.98± 0.73	0.27±0.16	10.97±6.50	1.48±0.66

TABLE 5.4

RANGES OF PARAMETERS CALCULATED FOR MACRONEKTON

	Muscle	Liver	Gonads
Total Alkanes (ppm)	0.02-2.4	1.1-28.1	2.3-98.6
Pristane/Phytane Ratio	0.0-115.0	4.9-166.8	6.6-234
Pristane/C_{17}	0.5-42.4	0.4-36.8	0.3-42.4
Phytane/C_{18}	0.5[a]	0.1-4.0	0.1-1.5
CPI_{14-20}	1.0-32.9	1.3-125.8	1.0-19.5
CPI_{20-32}	0.2-16.1	0.9-2.3	0.9-1.7

[a] Only 1 level found.

The C_{15} and C_{17} hydrocarbons were the dominant n-alkanes. Pristane was present in almost all samples. Phytane was present in approximately 10% of the muscle samples and in all liver and gonad samples. Few aromatic compounds were detected in these samples; those present were at levels too low for quantitation. Squalene was the dominant hydrocarbon in the aromatic fraction.

Analyses of Possible Shipboard Contaminants

Samples of oily bilge water, a ship's lubricant and a fuel oil were analyzed by techniques similar to that used for biota. These substances yielded characteristic petroleum-like patterns of aliphatic hydrocarbon distribution with no odd-even predominance. Aromatics were found in the appropriate samples, *e.g.*, the fuel oil. The results of these analyses are summarized in Tables 41 and 42, Appendix D.

DISCUSSION

General Comments

The overall purpose of this study was to provide basic data for hydrocarbons in biota and to evaluate if the hydrocarbons detected were of biogenic or anthropogenic origins. As the hydrocarbon concentrations in biota are generally very low, the avoidance of contamination during sampling and analysis is essential. The good blanks, recoveries and reproducibilities of this study along with the absence of evidence of ship's contaminants in the samples demonstrated that adequate precautions were taken. The analytical methods used were also sufficiently sensitive for this study as shown by the fact that the analysis of larger samples did not yield additional information. The data obtained from this study were similar to that obtained in previous years. The total concentrations of aliphatic hydrocarbons in muscle were in the low parts per million (μg/g dry weight) with n-pentadecane, n-heptadecane and pristane being the dominant hydrocarbons. Aromatic compounds were found at very low levels, generally from 5 to 30 parts per billion (ng/g dry weight). Overall, the data suggested very little, if any, petroleum contamination of the study area.

Macroepifauna

Analyses were carried out on 109 macrobenthic samples collected seasonally, and on 36 intraspecific variability and five 10-kg samples collected opportunistically. These data are summarized in Tables 1-16, Appendix D. As in the past, sampling problems yielded a rather wide diversity of species, making data correlations difficult. In addition, the absence of laboratory studies on the effects of low-level, chronic exposure to petroleum made it difficult to assess which parameters accurately indicated the presence of petroleum. Thus, although some trends could be inferred, it was difficult to draw any definite conclusions from the data.

Two parameters that are generally associated with petroleum pollution are the presence of phytane or an unresolved complex mixture (UCM)(Farrington *et al.*, 1972). Unresolved complex mixtures occurred in 56 of the samples while phytane occurred in 30; they occurred together in 29 samples. Station 1/IV had the most samples with phytane and a UCM, with Stations 1/I, 2/II and 2/IV having the highest frequency of these parameters. These results were suggestive of contamination from onshore and shipping activities; further studies are needed to confirm this hypothesis. As the levels found were very low (ppb) and as organisms tend to concentrate hydrocarbons when in contaminated water (Anderson *et al.*, 1974; Neff *et al.*, 1976), these findings suggested very low levels of petroleum hydrocarbons.

The carbon preference indices (CPI) have also been used as a measure of petroleum contamination (Clark, 1974). These ratios generally yield low, consistent numbers for petroleum and high, varied numbers for biological hydrocarbons due to the odd-carbon dominance found in organisms. These parameters were calculated as follows:

$$CPI_{14-20} = 1/2 \left(\frac{\sum_{n=15}^{n=19} \text{HC odd}}{\sum_{n=16}^{n=20} \text{HC even}} + \frac{\sum_{n=15}^{n=19} \text{HC odd}}{\sum_{n=14}^{n=18} \text{HC even}} \right)$$

$$CPI_{20-32} = 1/2 \left(\frac{\sum_{n=21}^{n=31} \text{HC odd}}{\sum_{n=22}^{n=32} \text{HC even}} + \frac{\sum_{n=21}^{n=31} \text{HC odd}}{\sum_{n=20}^{n=30} \text{HC even}} \right)$$

The CPI_{20-32} is generally of the same order of magnitude for petroleum (mean 1.2) and for biological organisms (mean 1.0-1.5), but the CPI_{14-20} more accurately reflects the odd-carbon dominance of biological samples that is absent in petroleum. The CPI_{14-20} is almost always greater than two for organisms, and averages less than one for petroleum (Clark, 1974). In this study, fairly consistent CPI values were found within species, as shown in Table 5.5. In some cases, such as shrimp, the CPI value appeared to normally be less than two. In most cases, low CPI values did not correspond with the presence of phytane or an unresolved complex mixture in indicating the presence of petroleum. This was probably due to the dominance of the C_{15} and C_{17} hydrocarbons which tended to yield high-CPI values and thus possibly masked the effect of petroleum on the CPI. These hydrocarbons were probably present at high levels (often 70% or more of the hydrocarbon concentration) due to the diet of the organisms; the C_{15} and C_{17} n-alkanes are the dominant hydrocarbons in algae (Clark and Blumer,

TABLE 5.5

RANGE OF CPI_{14-20} VALUES IN SELECTED SPECIES

Species	Number of CPI_{14-20} Values Number Sampled	Range of CPI_{14-20} Values
Penaeus aztecus (Brown shrimp)	11/16	0.8-3.6
Pristipomoides aquilonaris (Wenchman)	10/11	6.4-16.9
Stenotomus caprinus (Longspine porgy)	8/9	1.3-9.3
Micropogon undulatus (Atlantic croaker)	5/5	1.9-5.0
Cynoscion arenarius (Sand trout)	4/5	6.9-31.2

1967). In this study, the CPI ratios did not indicate the presence of significant petroleum contamination in the study area.

There was also an absence of correlation between and within stations of the pristane/phytane, pristane/C_{17} and phytane/C_{18} ratios. These parameters are often used to identify sources of oil pollution and would be expected to be similar in organisms exposed to a single petroleum source, although there is some indication that biogenic hydrocarbons can affect the ratios (Farrington and Medeiros, 1975). The lack of correlation of the ratios, however, further implied the absence of significant petroleum sources in the STOCS. The very low levels of aromatic compounds detected also suggested that there was very little petroleum pollution in the study area.

Macronekton

Only 54 of the 72 contracted macronekton analyses were performed due to the unavailability of samples. Specifically, only two (of four contracted) fall and winter samples were received and the two March samples were not received. (Each sample yields three analyses, thus the six missing samples produced a loss of 18 analyses.) The samples were red and vermilion snapper and yielded fairly consistent hydrocarbon profiles. The range of values calculated for the samples is shown in Table 5.4, while the hydrocarbon distributions are detailed in Tables 7-10, Appendix D. The dominant hydrocarbons were the C_{15} and C_{17} *n*-alkanes and pristane. Phytane was present in only 2 of 18 muscle samples, but was present in all liver and gonad samples. An unresolved complex mixture was detected in the chromatograms of almost all samples. The significance of this finding was difficult to ascertain, as the very low levels of aromatic compounds and the calculated parameters were not suggestive of the presence of petroleum in the study area.

CONCLUSIONS

As in the previous years of this study, the hydrocarbons detected in biota were of mainly biogenic origin. Occasionally, some evidence of hydrocarbons of petroleum origin was found, but the levels were very low suggesting very little contamination of the study area. The hydrocarbon profiles for a large number of species were obtained. These profiles provided a wide data base for determining the effects of petroleum exploration and production on the hydrocarbon content of biota from the STOCS.

LITERATURE CITED

Anderson, J. W., J. M. Neff, B. A. Cox, H. E. Tatem, and G. M. Hightower. 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. 27:75-88.

Clark, R. C., Jr. 1974. Methods for establishing levels of petroleum contamination in organisms and sediment as related to marine pollution monitoring. NBS Spec. Publ. 409. Marine pollution monitoring (petroleum). Proceedings of a symposium and workshop. Gaithersburg, Md. pp. 189-194.

Clark, R. C., Jr., and M. Blumer. 1967. Distribution of paraffins in marine organisms and sediment. Limnol. Oceanog. 12:79.

Farrington, J. W., C. S. Giam, G. R. Harvey, P. L. Parker, and J. Teal. 1972. Analytical techniques for selected organic compounds. Marine pollution monitoring: strategies for a national program workshop sponsored by NOAA at Santa Catalina Marine Biological Laboratory. October 25-28. pp. 152-176.

Farrington, J. W., and G. C. Medeiros. 1975. Evaluation of some methods of analysis for petroleum hydrocarbons in marine organisms. Proceedings of the 1975 conference on prevention and control of oil pollution, San Francisco, Ca. pp. 115-121.

Giam, C. S., and M. K. Wong. 1972. Problems of background contamination in the analysis of open-ocean biota for chlorinated hydrocarbons. J. Chromatog. 72:283-292.

Giam, C. S., H. S. Chan, and G. S. Neff. 1975. A sensitive method for the determination of phthalate ester plasticizers in open-ocean biota samples. Anal. Chem. 46:2225-2228.

Neff, J. M., B. A. Cox, D. Dixit, and J. W. Anderson. 1976. Accumulation and release of petroleum-derived aromatic hydrocarbons by four species of marine animals. Mar. Biol. 38:279-289.

CHAPTER SIX

TRACE METALS IN EPIFAUNA, ZOOPLANKTON AND MACRONEKTON

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ABSTRACT

Concentrations of 10 metals (Al, Ca, Cd, Cr, Cu, Fe, Ni, Pb, V, Zn) were determined in 340 samples including: zooplankton (60); muscle tissue from fish (52) and shrimp (19); fish gill (8) and liver tissue (115); and shrimp hepatopancreas (86). Samples were collected from Stations 1-3, all transects, Southern Bank and Hospital Rock during 1977. Concentrations were determined by flame atomic absorption spectroscopy (AAS)(Cu, Fe, Zn), flameless AAS (Cd, Cr, Ni, Pb) and neutron activation analysis (Al, Ca, V).

In 1977, fewer species (8) were analyzed than in the previous two years so that the number of replicate analyses of selected samples collected at the same place and time could be significantly increased. Replicate analyses of primarily liver and hepatopancreas tissue from individual organisms accounted for 59% of the total analytical effort. These tissues were selected because they contain detectable levels of all metals studied. An average of four replicate samples of these tissues were analyzed for each fish and shrimp sample collected.

No indication of substantial heavy metal pollution was observed. Sample groups in order of decreasing total trace metals content (except Al, Ca, V) were zooplankton, liver, hepatopancreas, gills, shrimp and fish muscle. No significant changes in annual mean trace metal concentrations were found for any sample group between 1976 and 1977.

The amount of variability in trace metal concentrations among the major sample groups in 1977 was similar to that observed in 1976. Groups in order of decreasing variability were zooplankton, liver, hepatopancreas, shrimp muscle, fish muscle and gills. Variability within species was only moderately less than that in groups. However, intrastation variability for zooplankton, liver and hepatopancreas samples as determined by replicate analyses was less than half that of the groups. With this level of variability, a 50% difference in the trace metal levels between two stations could be detected statistically ($p < 0.05$) on the basis of 5-10 replicate analyses at each station.

For zooplankton samples, Pb concentrations decreased offshore, and Cd levels showed the reverse trend. The levels of all metals except Cd were highest in the fall season.

Fish and shrimp muscle had generally low, uniform trace metal levels with few apparent geographical, seasonal or interspecific differences.

Fish species in order of decreasing total content of trace metals (except Al, Ca, V) were *Rhomboplites aurorubens*, *Stenotomus caprinus*, *Pristipomoides aquilonaris*, *Lutjanus campechanus*, and *Serranus atrobranchus*. Few significant correlations between liver trace metal concentrations and fish length were observed. Levels of Cd, Ni, and Pb were generally highest in livers of *S. caprinus* collected from the northern half of the STOCS study area.

Gill and liver tissue from *R. aurorubens* had generally higher levels of trace metals than similar tissues from *L. campechanus*. Cadmium levels were higher in livers than gills for both species. Within each species, the concentrations of all metals studied were similar for both bank stations.

Both species of shrimp analyzed had similar levels of trace metals in their hepatopancreatic tissue. This tissue contained the highest Ni levels of any sample type analyzed. Cadmium levels were similar to those in fish livers. Cadmium and Ni levels were maximal in the winter season. Significant differences between concentrations of various trace metals at different stations were observed, but no consistent geographical trends were apparent.

INTRODUCTION

This report covers the third (1977) and final year of the South Texas Outer Continental Shelf (STOCS) benchmark study for trace metals in epifauna, zooplankton and macronekton. The purpose of this project was to establish a data base on trace metal concentrations in STOCS organisms before extensive oil and gas exploration and production begins. The collection of this data would then provide decision-makers with the ability to assess the impact of future drilling/production activities in terms of changes in organismal trace metal levels. To assess the geographical variability of trace metals in STOCS biota, a variety of organisms were collected each year from Stations 1-3 on each of the four transects and from several topographic features (fishing banks). All stations except a few bank stations were sampled at least three times a year to determine any seasonal effects on trace metal levels.

During the first two years of this study (1975, 1976) trace metal levels were determined in a variety of organisms from the STOCS area. Replicate analyses of the same tissue from samples of the same species collected during the year were limited. Only single pooled samples of tissue from organisms collected at the same station and time were analyzed. Essentially no replicate analyses of tissues from individual organisms of the same species collected at the same station and time were conducted. The resulting data sets gave only a general picture of metal levels in STOCS biota. Broad geographical or seasonal trends could only be analyzed statistically by grouping similar samples in various ways to get an estimate of the variability in trace metal concentrations. The variability in such grouped data was high and very few significant relationships were observed. Comparisons between individual stations were not possible.

In an effort to reduce variability in the trace metals data and permit comparisons between individual stations, the number of species analyzed in 1977 was drastically reduced (*i.e.*, 29 in 1976; 8 in 1977). Replicate analyses of liver or hepatopancreas tissue from individual organisms collected at the same time and station were emphasized. These tissues were chosen because they contained measurable levels of most of the metals of interest. Many of the sample types (*e.g.*, fish muscle, shrimp muscle) analyzed in previous years contained levels of several trace metals that were below the detection limits of even flameless AAS analysis. This approach provided estimates of intra-station variability in trace metal levels. Such estimates were essential to statistically compare trace metal levels observed at different stations and to determine sample size requirements for any future monitoring program.

The purpose of this report is to characterize the 1977 trace metals data in terms of mean concentrations in the various types of organisms analyzed and to compare these levels with similar data from 1976 and 1975. The geographical and seasonal trends in 1977 organismal trace metal concentrations are discussed for each sample type and for individual species. The nature and magnitude of possible future changes in STOCS organismal trace metals levels that could be detected using the current data set is evaluated. Finally, the sampling regime which should be employed in any future STOCS monitoring program is discussed.

MATERIALS AND METHODS

All samples were collected by personnel of the University of Texas, Port Aransas Marine Laboratory. Shrimp and demersal fish samples were collected with a 35 ft (10.7 m) Texas box otter trawl. Zooplankton samples were collected with a metal-free, 1 m, 220 μm mesh net. Macronekton samples were caught by hook and line. Subsurface suspended patriculate matter samples were taken using 20-ℓ acid-cleaned polyethylene carboys. Every reasonable precaution was taken to avoid contamination during sampling. Shrimp, demersal fish and macronekton samples were placed in polyethylene bags. When potentially contaminating sediment of other foreign material was adhering to the exterior surfaces of the organisms collected, they were rinsed prior to being put into the polyethylene bags. Zooplankton samples were transferred from the cod-end of the net to plastic snapcap vials. During this procedure a representative aliquot of each zooplankton sample was taken and preserved in buffered formalin for later taxonomic analysis by Dr. Park's laboratory. To avoid any release of metals from the organisms caused by microbial activity, all samples (until prepared for analysis) were immediately frozen on board ship and remained frozen during transportation and storage. To minimize the growth of phytoplankton and other microorganisms in the particulate matter samples, each sample was poisoned with chloroform immediately after collection. These samples were kept refrigerated in the dark until preparation for analysis.

Epifauna and demersal fish sampling trawls were made at Stations 1-3, all transects, during the three seasonal sampling periods. However, to permit more intra-station replicate analyses, only six selected species were taken from these trawls for trace metal analysis. The species selected were *Penaeus aztecus* and *P. setiferus* (shrimp), *Pristipomoides aquilonaris, Serranus atrobranchus, Stenotomus caprinus* and *Trachurus lathami* (demersal fish). These species were chosen because of their ecological and/or commercial importance within the STOCS region and because of their frequent occurrence in collections from the first two years of this study. Whenever any of these species were present in the trawl catch at any station, at least five individuals were sampled. Samples were not collected at all twelve stations during each of the three seasons due to variability in the occurrence of these species at the sampling sites. A total of 63 shrimp and demersal fish samples were collected during the three seasonal sampling cruises. The number of samples of each species collected during 1977 is detailed in Table 6.1. At least one species sample was collected at 10 of the 12 sampling stations during the year. Only the two, northern, inshore stations (1/I and 1/II) failed to yield any samples of the selected species during 1977.

Macronekton samples were also collected from Southern Bank and Hospital Rock during the three seasonal sampling periods. Optimally five individuals of two species (*Lutjanus campechanus* and *Rhomboplites aurorubens*) were collected from each bank during each sampling season. A total of eight macronekton samples were collected as summarized in Table 6.1.

Zooplankton samples were collected from Stations 1-3, all transects during the three seasonal sampling periods (Table 6.1). Sixty (60) zooplankton samples were collected including 12 samples taken in triplicate.

Suspended particulate matter samples were collected from Stations 1 and 3 on each of the four transects during the winter and spring sampling seasons.

Sample Preparation

Non-contaminating procedures used to prepare each of the major sample types (*i.e.*, zooplankton, shrimp muscle and hepatopancreas, fish muscle, liver and gills) are detailed below. Experiments conducted during the 1976 study (Presley and Boothe, *In* Groover, 1977) showed that these procedures made no detectable contribution to the levels of the eight metals of interest observed in the resulting samples.

TABLE 6.1

ANALYSES BY SAMPLE TYPE, SPECIES AND COLLECTION DATA

	Species collection data			
Sample type and species	Number of Seasons Sampled	Number of Transect Stations Sampled	Number of Bank Stations Sampled	Number of Samples Analyzed
Zooplankton (ZPL)	3	12	-	60
Macroepifauna (EPI)				
A. Shrimp - muscle (tissue pooled from ≤ 10 individuals)				
1. Penaeus aztecus	3	9	-	15
2. Penaeus setiferus	2	3	-	4
B. Shrimp - hepatopancreas (tissue from individual shrimp)				
1. Penaeus aztecus	3	9	-	67
2. Peneaus setiferus	2	3	-	19
Demersal fish (EPI) and macronekton (MNK)				
A. Muscle (tissue pooled from ≤ 10 individuals)				
1. Lutjanus campechanus	3	-	2	4
2. Pristipomoides aquilonaris	3	5	-	14
3. Rhomboplites aurorubens	3	-	2	4
4. Serranus atrobranchus	3	8	-	11
5. Stenotomus caprinus	3	6	-	11
6. Trachurus lathami	2	3	-	8
B. Gills (tissue pooled from ≤ 5 individuals)				
1. Lutjanus campechanus	3	-	2	4
2. Rhomboplites aurorubens	3	-	2	4
C. Livers (tissue pooled from ≤ 5 individuals)				
1. Lutjanus campechanus	3	-	2	4
2. Rhomboplites aurorubens	3	-	2	4
D. Livers (tissue from individual fish)				
1. Lutjanus campechanus	3	-	2	18
2. Pristipomoides aquilonaris	3	5	-	39
3. Rhomboplites aurorubens	3	-	2	20
4. Serranus atrobranchus	1	1	-	3
5. Stenotomus caprinus	3	6	-	27
			Total	340

No sorting was made of the diverse and variable group of zooplankton organisms collected. Each sample was thawed and poured into an acid-cleaned 140 mm diameter plastic petri dish. Filtered seawater was added as necessary to cover the entire sample. The sample was carefully inspected using a dissecting microscope. Any foreign material was removed and the relative proportion of major types of zooplankton (*e.g.*, copepods, ostracods, crustacean larvae, chaetognaths, etc.) in the sample was estimated. The sample was poured onto a 220 µm NITEX screen and rinsed sparingly with deionized water. The deionized water used for all work in this study was prepared by passing distilled water through an ultrapure, mixed-bed demineralizer column (BARNSTEAD D0809). Excess water was removed by placing the screen over a series of paper towels and gently squeezing the sample with a teflon spatula; the sample was weighed immediately to determine wet weight. The vial was covered with parafilm and placed in a freezer until it could be freeze-dried.

Fish and shrimp samples were thawed just prior to being prepared for freeze drying. They were rinsed with deionized water as necessary to remove any mud or other foreign material adhering to the exterior surfaces of the organisms. All dissections were done in a clean room on acrylic plastic cutting boards using stainless steel scalpels, scissors and nylon or teflon tweezers as required. At no point during the dissection were the preparer's fingers allowed to touch the tissue to be analyzed. All dissecting equipment was thoroughly rinsed with 1 N nitric acid (HNO_3) and deionized water between each sample. At the end of each preparation session, all equipment was thoroughly cleaned in a Na_2CO_3 solution and rinsed with 1 N HNO_3 and deionized water. The equipment was stored in polyethylene bags until the next use. The acrylic boards were soaked in 0.5 N HNO_3 between each use.

Muscle tissue from all fish and shrimp collected was prepared for analysis. A maximum amount of tissue from each individual was prepared for freeze drying as described below. This action was taken to insure that extra freeze dried material would be available for repeat analyses when necessary and to avoid having material from the same sample stored in two different ways for long periods. An equivalent wet weight tissue aliquot was taken from up to 10 individuals in the sample (if available) and pooled in a tared plastic, snap-cap vial to give a total wet weight if possible of 6-12 g. After dehydration this pooled sample yielded a dry weight of 1-3 g, all of which was analyzed for trace metals. Pooled muscle tissue samples were prepared in this manner to insure that the trace metals concentrations in the pooled sample represented a true average of the concentrations existing in each of the individual organisms included in the sample and also to avoid having to homogenize a large, pooled sample with a ball mill or mortar and pestle and risk contamination. If sufficient tissue remained, reserve pooled samples identical to the first were prepared in separate vials. If there was insufficient tissue remaining to prepare a second replicate pooled sample, or if there was still tissue left over after the preparation of the additional replicates, the remaining tissue from each individual was placed in separate vials for possible future use. Pooled samples of gill tissue from each *Lutjanus campechanus* and *Rhomboplites aurorubens* sample were prepared in this same manner.

The hepatopancreas was removed from the body cavity of each shrimp for analyses. The excision was accomplished while the shrimp was still partially frozen. This approach minimized the problems associated with removing such a friable organ intact. After removing the hepatopancreas, the total length (from rostrum to tail) and sex (whenever possible) were determined for each individual. The head and thorax were then cut off and discarded. The abdominal musculature was removed by making a mid-ventral incision with scissors and peeling off the exoskeleton. The mid-ventral artery was removed from the surface of the muscle and the digestive tract and dorsal artery excised by making a mid-dorsal incision. This procedure was done to reduce the variability in sample trace metal concentration since vascular and digestive tissue could have significantly different trace metals content than the muscle tissue. The muscle tissue was rinsed sparingly with deionized water to remove any remnants of the arteries or digestive tract and was then handled as described below.

The standard length and sex (whenever possible) of each individual fish were determined. In dissecting out the lateral trunk musculature, a concerted effort was made to avoid contamination of the muscle sample which could occur if the sample came into contact with the exterior surface of the skin. On each side of the fish, a dorso-ventral incision was made along the anterior margin of the lateral trunk musculature. This incision was continued posteriorly just lateral to the mid-dorsal and mid-ventral planes. The skin was flayed off and discarded. The muscle was cut away from the axial skeleton, and, when sufficient tissue was available, the margins, where possible contact with the exterior skin could occur, were trimmed off and discarded. If there was insufficient tissue available these margins were rinsed sparingly with deionized water. For macronekton samples, gill tissue were also sampled. The gills (including gill rakers) were removed by cutting the dorsal and ventral attachments and were rinsed sparingly with deionized water to remove any foreign material. Pooled samples of gill tissue were prepared as described above. The liver was removed from most fish for analysis. Initial work showed that because of their small size too little liver tissue was available from individual *Serranus atrobranchus* and *Trachurus lathami* to permit routine trace metal analysis. Thus, analyses on the liver tissue from individual demersal fish were restricted to two species (*Pristipomoides aquilonaris* and *Stenotomus caprinus*). In some cases even with these species, it was necessary to pool liver tissue from several individuals to achieve a dry weight (> 0.1 g) sufficient for analysis. Pooled liver tissue samples from each macronekton sample (*Lutjanus campechanus* and *Rhomboplites aurorubens*) were analyzed. These pooled samples were prepared by mixing equal dry weight aliquots of homogenized liver tissue from each individual fish of a species collected at the same time and place. Because of the relatively large livers in these species, it was also possible to analyze separately samples of liver tissue from each individual fish of both species collected.

At the end of each sample dissection, the tissue sample(s) was placed immediately in a tared snap-cap vial and weighed immediately to determine wet weight. The samples were covered with parafilm and placed in a freezer. When a sufficient number of samples had accumulated, all samples were freeze-dried for 24 to 96 hours to a constant weight. After removal from the freeze dryer, the samples were reweighed to determine dry weight and the percentage of moisture lost by each sample was calculated. Samples were then stored in a desiccator until analyzed. The following experiment was conducted to confirm the assumption that trace metals were not being lost from the samples during freeze-drying. Three pairs of duplicate fish muscle samples were prepared. One sample of each pair was prepared for analysis in the normal way. The second sample was handled in the same manner except it was digested wet without first being freeze-dried. No significant differences in trace metal levels were observed between any of the three pairs of samples. Any error involved in converting wet weight to dry weight concentrations for comparisons was negligible.

Suspended particulate matter samples were allowed to gravity settle for several months while refrigerated in the dark. The top 18 ℓ of each sample was then carefully removed by siphoning using acid-cleaned teflon tubing. The remaining 1-2 ℓ was vigorously shaken and filtered through a tared 0.5 μm fluoropore filter. These filters were carefully desiccated in non-contaminating containers and the weight of each determined to the nearest microgram before use. All filtrations were conducted in a clean room using acid-cleaning glassware to avoid particulate contamination. The filter is composed of PTFE teflon on a high density polyethylene backing and was chosen because it could be leached in a hot acid solution without decomposing. After use the filters were placed in the same non-contaminating containers, desiccated in the same manner and reweighed. The total weight of material collected on the filters ranged from 1.57-92.8 mg. The filters were then leached individually in acid employing the same wet oxidation procedure used to prepare organism samples for AAS analysis (see the next section). The only exception was that before the acid solution was evaporated to near dryness the filters were removed with non-contaminating tweezers and rinsed with 0.1 N Ultrex HNO_3. The concentrations of the eight metals in the resulting sample solutions were determined by flameless AAS analysis. This approach was used to evaluate which metals of interest associated with suspended matter would be released in significant amounts when organisms with incorporated particulate matter were digested.

Digestion (Wet Oxidation) of Samples

Freeze-dried samples were prepared for atomic absorption spectrophotometric (AAS) analysis using a nitric (HNO_3) perchloric ($HClO_4$) acid digestion procedure described in Method 3 of Attachment B to the 1977 BLM contract. This procedure, as used in our laboratory, yielded very acceptable procedural blanks which are summarized later in this section. In this procedure the volume of acids used was minimized by employing an essentially closed refluxing system during the digestion process. A 1-3 g dry weight sample was placed in a spoutless, electrolyte style pyrex beaker and 4-5 ml of 70% HNO_3 per gram of sample and 1 ml total of $HClO_4$ were added. The beaker was covered with a 75 mm, non-ribbed pyrex watchglass and allowed to sit overnight at room temperature. The mixture was then refluxed at low heat on a hotplate for 6-24 hours. A bent glass rod was placed between the beaker lip and the watchglass and the heat increased to permit HNO_3 evaporation. At the first sign of white $HClO_4$ fumes (*i.e.*, when most of the HNO_3 was gone), the glass rod was removed allowing the watchglass to again rest flush on top of the beaker. The sample was allowed to reflux until cleared completely. If the sample did not clear, an additional 1 ml HNO_3 and 0.5 ml $HClO_4$ was added and the refluxing continued until clearing occurred. This step was repeated once if necessary. Finally, the watchglass was removed and the mixture was allowed to evaporate to near dryness. Spike recovery experiments conducted frequently during the 1976 study showed that there was no significant loss of any of the metals studied during this digestion procedure (Presley and Boothe, *In* Groover, 1977).

Each digested sample was transferred to a tared 30 ml Oak Ridge-type, screw-top polypropylene centrifuge tube by washing the beaker several times with 0.1 N HNO_3 (BAKER ULTREX grade) and pouring the resultant solutions into the centrifuge tube. This transfer procedure was apparently quite complete. Randomly selected beakers were occasionally rinsed with stronger acid (1 N HNO_3) after the sample had been removed to determine the amount of metals remaining. This acid solution was then analyzed using our routine AAS procedures. Even for samples such as zooplankton and fish livers which contain relatively high levels of the trace metals studied, the residual amounts of metals in the digestion beakers were minimal (*i.e.*, << 1%). Each sample was brought to 15-25 ml, thereby diluting the original dry weight sample 10-30 times. The volume of each sample was determined by reweighing the filled sample tube. No correction for the specific gravity of the sample solution was necessary because this value was very close to 1.0 (*i.e.*, averaged < 1.03). Insoluble residue which occurred in significant amounts in several zooplankton and fish gill samples was allowed to gravity settle in the tubes. Further dilutions from the original solution were made on a weight/weight basis in presoaked 5 dram snap-cap vials using 0.1 N HNO_3.

All digestion glassware was soaked immediately after use in a solution of "Micro" detergent and distilled water in covered polyethylene pans for up to several days. The glassware was then rinsed thoroughly with deionized water and soaked in 3 N reagent grade HNO_3 in covered polyethylene or polypropylene pans until the next use. The centrifuge tubes were prepared for use by cleaning in a "Micro" solution. They were then filled with 5 N reagent grade HNO_3, heated for several days at 50°C and stored at room temperature until used. Prior to use, the tubes were emptied, rinsed thoroughly with deionized water and tared. The 5 dram snap-cap vials used for further dilutions were filled with 1 N reagent grade HNO_3 and allowed to sit at room temperature for several days. Prior to use they were emptied, rinsed with deionized water and tared.

About 50 samples and blanks were digested at any one time using the above procedure. Three to five procedural blanks were included in each digestion to determine the amount of each metal contributed to the samples by the digestion glassware and reagents. These blanks received the same reagents and treatment as the tissue samples. An aliquot of the 0.1 N HNO_3 used to transfer and dilute the sample was placed in a centrifuge tube and analyzed with each digestion as a diluent/tube blank. Reagent blanks were analyzed for all bottles of acid prior to their use in sample digestion. These blanks were prepared by taking $\geq$ 10 ml of acid, evaporating it to near dryness in digestion glassware and transferring the residue to a centrifuge tube in the same manner described above. For each series of dilutions made using 5 dram vials, one or more vial blanks were prepared and analyzed.

Atomic Absorption Spectroscopy (AAS) Procedures

Eight elements (Cd, Cr, Cu, Fe, Ni, Pb, V and Zn) were analyzed in biological samples from the 1977 STOCS study. Cadmium, Cr, Ni and Pb, which occurred at low levels, were measured using flameless AAS. These analyses were made using a PERKIN-ELMER Model 306 atomic absorption spectrophotometer equipped with an HGA-2100 graphite furnace atomizer. A summary of the instrumental operating conditions and the average procedural blanks for all eight digestions are given in Table 6.2. External and internal furnace purge gas flow rates were verified at specified levels of 0.9 and 0.3 ℓ per minute respectively at 40 psi delivery pressure. Injection volume was 25 µl. The furnace temperature gauge was calibrated using a clamp-on (inductive) ammeter and an optical pyrometer. Dry, char and atomization temperatures and times were optimized for each metal using selected representative samples according to the manufacturer's recommendations (Anon., 1974). Non-resonance lines used for this optimization to estimate the magnitude of broad bank molecular absorption for various sample types were 226.5 (Cd), 231.6 (Ni), 282.0 (Pb) and 352.0 (Cr) nm. Corrections for non-specific or broad bank molecular absorption were made by a deuterium arc background corrector. For Cd and Pb, sample dilutions

TABLE 6.2

SUMMARY OF OPERATING CONDITIONS FOR FLAMELESS ATOMIC ABSORPTION ANALYSIS

Element	Wavelength (nm)	Source[1]	Temperature (°C) Time (seconds) dry	char	atomize	Minimum Detectable Concentration[2] (ppb)	Sensitivity[3] (pg)	Average Total Procedural Blank[4] (ng)
Cd	228.8	EDL (5) HCL (8)	85° 60 sec	300° 60 sec	1800° 8 sec	0.025	9	< 2
Cr	357.9	HCL (10)	85° 60 sec	800° 30 sec	2600° 8 sec	1	25	20
Ni	232.0	HCL (20)	85° 60 sec	1200° 30 sec	2500° 8 sec	4	100	<90
Pb	283.3	EDL (9)	85° 60 sec	500° 60 sec	2000° 8 sec	0.5	25	18

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[1] Electrodeless discharge lamp (EDL). Numbers in parentheses are source energy in watts. Hollow Cathode Lamp (HCL). Numbers in parentheses are source current in milliamps.

[2] At 10x scale expansion and approximately 1 chart unit; except Ni at 3x and 2 chart units.

[3] Average amount of metal injected giving a signal of .0044 absorbance units.

[4] Total nanograms of element added to sample by reagents and labware used in sample preparation.

$\geq$ 1/50 were used for quantitation, and for Cr and Ni, dilutions of $\leq$ 1/50. Chemical interference was evaluated and corrected as necessary by frequent use of the standard additions technique and check dilutions. Mixed standard metal solutions were prepared in dilute HNO_3 (BAKER ULTREX grade) by diluting concentrated commercial atomic absorption standards. Samples were quantitated by peak height comparison with bracketing standards injected before and after the sample. Consideration was given to temporal variations in instrumental sensitivity, non-linearity between bracketing standards and gross differences in peak shape.

Copper, Fe and Zn were analyzed by flame AAS using a JARREL-ASH Model 810 atomic absorption spectrophotometer. Analyses were carried out following the manufacturer's recommended procedure (Anon., 1971; 1972). A summary of the operating parameters for these analyses is given in Table 6.3. Non-specific absorption was monitored by measuring simultaneously the absorbance of a non-resonance line and the analytical line of the element of interest. A lean air-acetylene flame with flow rates of circa 7 and 2.5 ℓ per minute, respectively, were used for all three elements. Aspiration rate was generally 5 to 6 ml per minute. Chemical interference was checked by use of the standard additions technique. Mixed standards used were prepared as described above.

The accuracy and precision of AAS analysis was evaluated by analyzing a National Bureau of Standards (NBS) standard biological reference material (*i.e.* #1577 bovine liver) with each digestion. The results of these analyses compared to NBS certified values are given in Table 6.4. Why our average Fe concentrations is consistently below the NBS value is not known. Several different batches of Fe standards were used to determine these various concentrations during the course of this study. A locally prepared fish muscle house standard was also analyzed with each digestion to estimate

TABLE 6.3

SUMMARY OF OPERATING CONDITIONS FOR FLAME ATOMIZATION ATOMIC ABSORPTION ANALYSIS

Element	Analytical Wavelength (nm)	Non-resonance Wavelength (nm)	Sensitivity[1] (ppm)	Average Total Procedural Blank[2] (ng)
Cu	324.7	322.9	0.05	< 75
Fe	248.3	247.3	0.07	<100
Zn	213.9	220.2	0.02	< 75

[1] Average concentration giving a signal of .0044 absorbance units. Minimum detectable concentration was generally about one half of the sensitivity.

[2] Total nanograms of element added to sample by reagents and labware used in sample preparation.

TABLE 6.4

ACCURACY AND PRECISION OF ATOMIC ABSORPTION ANALYSIS

Standard Reference Material	Concentration (ppm dry weight ± 1 standard deviation)						
	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Bovine liver (NBS No. 1577)							
This study (n=7)	0.26 ± 0.02	0.09 ± 0.02	183 ± 3	232 ± 10	<0.08 ± 0.02	0.37 ± 0.05	130 ± 7
NBS values	0.27 ± 0.04	<0.2[1]	193 ± 10	270 ± 20	<0.2[1]	0.34 ± 0.08	130 ± 10
Precision[2]							
This study	6	26	2	4	23	14	5
NBS values	15	NA	5	7	NA	24	8
Fish muscle (House Standard)							
This study (n=8)	<0.01 ± 0	0.9 ± 0.4	1.2 ± 0.6	12 ± 5	0.29 ± 0.04	0.12 ± 0.06	19 ± 1
Precision[2]							
This study	0	39	45	44	15	49	6

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[1] Not certified values.

[2] Precision expressed as percent coefficient of variation i.e. (std. dev./mean) x 100.

the precision of AAS procedures at the very low metal levels observed in many of the sample types analyzed routinely (*e.g.* fish and shrimp muscle). These results are also summarized in Table 6.4. The precision of measurement decreased for several of the metals at low concentration, which would be expected. Cadmium was undetected in all eight of the replicate samples analyzed. Taken together these data indicate the AAS techniques used were acceptable.

Analysis of Vanadium in Organisms

As outlined previously, an effort was made this year to develop a chelating/co-precipitation procedure using ammonium pyrrolidine dithiocarbamate (APDC) which would improve the sensitivity of Vanadium (V) analysis in organisms by neutron activation analysis (NAA). Several separate recovery studies were conducted using a fish muscle sample matrix spiked with varying concentrations of stable $^{51}V^{+5}$ AA standard or $^{48}V^{+3}$ radiotracer. The sample pH and other parameters of the APDC procedure were varied in an effort to achieve an acceptably high and consistent yield. These experiments were not successful. Yields were generally < 60% and very inconsistent.

A possible explanation for this situation is that V can exist in a variety of different ionic species in solution. V^{+5} is probably the dominant oxidation state present in the highly oxidized organism digests. Cotton and Wilkinson (1972) list eleven pH dependent ionic species for V^{+5} in aqueous solution. At least three of these occur at the V concentrations and pH (*i.e.* 3-4) existent in the coprecipitation solutions. These three species can interconvert among themselves over this pH range. Differences in the distribution of V among these species in various samples plus any differences in the affinity of APDC for these species could be responsible for the variable and apparently very pH dependent yields observed.

Because of the above results, V concentrations in organisms were determined using the NAA method described in Attachment B to the 1977 BLM contract. In this procedure, sulfuric acid (H_2SO_4) and hydrated antimony pentoxide (HAP) were used as pre-irradiation chemical treatments to remove interfering chlorine (Cl) and sodium (Na), respectively, from acid digests of organism samples.

To remove Na, an aliquot of each organism digestate was diluted with an equal volume of 16 N HNO_3. The resulting solution was passed through a column of HAP according to the procedure of Girardi and Sabbioni (1968) as modified by Science Applications Inc. (Reed, 1977). Vanadium was not quantitatively eluted from this column. Corrections for incomplete recovery from the column were made on the basis of spiked replicate samples run with every group of samples. The antimony (Sb) carryover experienced by SAI was a continuing problem. However, careful manipulation of the counting parameters gave detection limits of 10-20 ng V which was adequate for most of the STOCS samples. Cl in the elutriate was removed by adding 0.2 ml of concentrated H_2SO_4 and evaporating the solution in a teflon beaker to near dryness or until SO_3 fumes were observed. The teflon beaker contents were poured into a 1.5 ml irradiation polyvial used by the Texas A&M University Nuclear Science Center. The vial was heat-sealed to prevent sample loss during analysis.

Each sample was irradiated separately for one minute by a 1 MW TRIGA REACTOR. This process was facilitated by a pneumatic transport system which could rapidly transfer samples in and out of the reactor core. Standards prepared from commercial AAS standards or pure metals were used.

After return of the sample and an appropriate delay period (usually two minutes, so that the dead time was < 30%), the irradiated sample was placed on an ORTEC Ge(Li) detector and counted using a separate GEOS Quanta

4096 channel pulse height analyzer. After a two minute counting period, the spectrum was stored on magnetic tape.

Data reduction was done using the program HEVESY (Schlueter, 1972). This program calculated peak intensities and converted them to concentration by comparison with standards. Corrections were made for varying delay times, dead times and neutron fluxes.

One characteristic of NAA was its capability for analyzing several elements from a single irradiation. Concurrent with V analysis, the concentrations of Al, Ca and Cu could be determined. However, the analytical conditions could not be optimized for all four elements during a single irradiation. The sensitivity for Al was good and the Al concentration data satisfactory. However, the sensitivity for Ca was marginal and many of the samples were below the detection limit which was quite variable and often very high. The sensitivity for Cu was very poor and almost all the samples were below the elevated detection limit.

RESULTS AND DISCUSSION

Appendix E contains the complete 1977 trace metals project data set. Aluminum, Ca and V data are missing from several samples for one of two reasons. First, Sb carryover in some samples prepared for NAA resulted in excessive background activity as discussed under methods. For a few samples insufficient sample remained after AA analyses to permit NAA. Table 6.1 summarizes the 340 samples analyzed according to the major sample groups during the 1977 study year: zooplankton (ZPL), macroepifauna and demersal fish (EPI), and macronekton (MNK). For the 1977 study these three groups were further divided into six groups of similar types of organisms or tissues: zooplankton, fish muscle, fish gill, fish liver, shrimp muscle and shrimp hepatopancreas. As discussed previously, analyses of a few selected species collected seasonally was the analytical approach used in the 1977 study. As a result certain sample types present in the 1976 STOCS data set are absent this year. Squid were not analyzed in 1977. Also, *Spondylus americanus* (Spiny oyster) and macronekton samples collected as part of the Topographic Features study are included in the fainal report for that project. Shrimp hepatopancreas tissue was added as a sample type in 1977 for reasons noted earlier.

This approach resulted in a significant increase in the number of replicate samples analyzed during the 1977 study. In 1976, 29 different species plus zooplankton were analyzed for trace metal content. This year samples from only eight species plus zooplankton were analyzed. Analyses of shrimp hepatopancreas tissue and fish livers accounted for 59% of the total analytical effort. For every sample of shrimp or fish collected more than four replicate samples of hepatopancreas or liver tissues were analyzed. These replicate samples are very important because they provided an estimate of the variability in trace metal levels within a species population at a single station. Such variability estimates were essential to statistically compare metal levels at different stations. Also, such estimates were needed to determine how many replicate analyses would be required in any future monitoring program to detect a given percentage difference in mean trace metal levels between different stations.

In 1975 and 1976 essentially no intra-station replicate analyses were done. To make temporal and spatial intraspecific comparisons within the STOCS study, data from individual species were organized into larger, meaningful groups. For purposes of comparing trace metal data from different years, the 1977 data were also grouped. The six groups selected for discussion were noted above. The zooplankton were a diverse group resulting from a specific sampling technique, and no sorting was done on the resultant samples. The fish muscle and liver groups included MNK samples from two bank stations and demersal fish (EPI) samples from the eight offshore transect stations (*i.e.* all stations 2 and 3). Gill samples were taken only from MNK samples taken at the two bank stations. Shrimp samples were collected at nine transect stations (except 3/I, 3/II, 3/IV) and thus was the group which had the largest geographical distribution.

Table 6.5 compares the annual mean concentrations of 10 elements in the above six groups with similar data from 1976 and 1975 analyses. These annual averages, although derived by grouping several similar species, provided a concise and general way of comparing differences in trace metal concentrations among different types of organisms and between the same kind of organism collected during different years. All averages in Table 6.5 were calculated using less-than values at the indicated limit of detection to avoid excluding too much data from consideration. Also, a few data points which were more than five standard deviations away from the annual averages were not used in the calculations to avoid unduly biasing these means. Few undetectable concentrations were observed in 1975 due to the systematic overestimation of the concentrations of several metals in many sample types for reasons discussed previously (Presley and Boothe, *In* Groover, 1977). Many more undetectable levels were observed in 1977 and 1976 due to the use of flameless AAS analysis. Fish muscle had the most frequent undetectable values for Cd, Ni and Pb and less frequently for Cr. Preliminary NAA work on 1977 fish muscle samples suggested that V levels in these tissues will again be generally low with many samples being below the detection limit of the analytical procedure. Nickel and Pb were generally below detection limits in shrimp muscle samples. Because less-than values were used in these calculations the true annual means for the above elements and sample groups were lower than indicated values. However,

TABLE 6.5

COMPARISON OF ANNUAL AVERAGES OF TRACE METALS IN MAJOR SAMPLE TYPES FROM 1975, 1976 AND 1977

Sample Type	No. of Species	No. of Samples	Year	Concentration (ppm dry weight ± 1 standard deviation)				
				Cd	Cr	Cu	Fe	Ni
Zooplankton (ZPL)	-	60	77	3.1 ± 1.5	3.8 ± 3.6	43 ± 87	3700 ± 4200	6.9 ± 5.8
	-	62	76	2.9 ± 1.4	4.3 ± 4.2	20 ± 22	2100 ± 3000	6.6 ± 6.2
	-	70	75	3.5 ± 1.4	4.4 ± 2.6	13 ± 7	-----	7.1 ± 3.4
Fish Muscle (EPI, MNK)	6	52	77	<0.01 ± 0	0.04 ± 0.02	1.2 ± 0.7	6 ± 5	<0.08 ± 0.02
	20	140	76	0.02 ± 0.02	0.04 ± 0.03	1.1 ± 0.5	6 ± 4	<0.09 ± 0.06
	16	118	75	0.11 ± 0.07	1.8 ± 1.3	1.2 ± 0.6	-----	1.0 ± 1.1
Fish Gills (MNK)	2	8	77	0.33 ± 0.21	0.09 ± 0.05	2.4 ± 0.7	260 ± 80	0.60 ± 0.12
	3	31	76	0.82 ± 1.2	0.12 ± 0.07	2.9 ± 2.5	140 ± 70	0.48 ± 0.14
	1	6	75	0.67 ± 0.25	3.8 ± 0.2	1.0 ± 0.4	116 ± 10	4.6 ± 0.4
Fish Livers (EPI, MNK: pooled and individual)	5	115	77	5.8 ± 5.6	0.10 ± 0.09	28 ± 22	820 ± 580	0.58 ± 0.76
	3	29	76	7.2 ± 8.5	0.07 ± 0.05	25 ± 23	850 ± 640	0.24 ± 0.22
	1	6	75	4.5 ± 1.4	2.2 ± 0.2	12 ± 2	560 ± 180	0.87 ± 0.10
Shrimp Muscle (EPI)	2	19	77	0.03 ± 0.03	0.04 ± 0.02	23 ± 4	5 ± 7	0.08 ± 0.02
	4	19	76	0.09 ± 0.08	0.04 ± 0.02	24 ± 9	4 ± 3	0.21 ± 0.14
	5	29	75	0.15 ± 0.07	1.8 ± 0.8	25 ± 4	-----	0.93 ± 0.66
Shrimp Hepatopancreas (EPI)	2	86	77	7.7 ± 4.1	0.45 ± 0.34	420 ± 320	250 ± 180	7.8 ± 5.7

TABLE 6.5 CONT.'D

Sample Type	No. of Species	No. of Samples	Year	Concentration (ppm dry weight ± 1 standard deviation)				
				Pb	Zn	V	Al	Ca
Zooplankton (ZPL)	–	60	77	16 ± 33	280 ± 340	14 ± 13	6,000 ± 7,500	40,000 ± 25,000
	–	62	76	20 ± 45	120 ± 170	13 ± 20	3,100 ± 5,500	35,000 ± 20,000
	–	70	75	11 ± 9	113 ± 38	17 ± 15		
Fish Muscle (EPI, MNK)	6	52	77	<0.04 ± 0.02	12 ± 4	<0.07 ± 0.03	19 ± 8.0	950 ± 950
	20	140	76	0.05 ± 0.05	12 ± 7	0.20 ± 0.30	25 ± 13	2,000 ± 5,500
	16	118	75	1.0 ± 0.5	16 ± 6	1.8 ± 1.4		
Fish Gills (MNK)	2	8	77	0.9 ± 0.7	85 ± 9	0.70 ± 0.60	55 ± 16	60,000 ± 30,000
	3	31	76	1.4 ± 1.6	82 ± 48	2.0 ± 2.0	110 ± 140	75,000 ± 40,000
	1	6	75	7.9 ± 1.8	64 ± 7			
Fish Livers (EPI, MNK: pooled and individual)	5	115	77	1.5 ± 3.4	190 ± 130	1.3 ± 1.5	45 ± 50	900 ± 650
	3	29	76	0.38 ± 0.36	320 ± 350	3.0 ± 6.0	80 ± 120	60,000 ± 70,000
	1	6	75	2.8 ± 2.4	160 ± 67			
Shrimp Muscle (EPI)	2	19	77	<0.05 ± 0.02	53 ± 9	0.11 ± 0.10	20 ± 20	1,300 ± 850
	4	19	76	0.08 ± 0.06	63 ± 18	0.50 ± 0.90	40 ± 40	1,800 ± 1,700
	5	29	75	0.86 ± 0.50	51 ± 9			
Shrimp Hepatopancreas (EPI)	2	86	77	0.25 ± 0.20	120 ± 36	4.5 ± 5.5	350 ± 1,100	2,500 ± 2,500

since the 1977 and 1976 detection limits for Cd, Cr, Ni and Pb were quite low (*i.e.* < 0.01 to 0.1 ppm dry weight), overestimation of these means should not be excessive and should not affect the relationships in these general comparisons.

Two approaches were used to characterize systematically the relative levels of trace metals (except Al, Ca, V) among the groups in Table 6.5. The first approach was to rank the groups numerically from one to six for each of the seven metals. The average ranking for each group was then determined (*i.e.* sum of ranks for each group/7). In 1977 zooplankton had generally the highest trace metals concentrations with shrimp hepatopancreas and fish livers having somewhat lower levels. The order of the remaining groups was fish gill, shrimp muscle and fish muscle.

The hepatopancreas and liver perform many of the same physiological functions in shrimp and fish, respectively. Both tissues accumulate similar levels of Cd and Cr. Lead levels were also generally similar in both tissues. However, significantly elevated Pb levels in *Stenotomus caprinus* liver tissue raised the mean Pb value for the fish liver group and masked this similarity. Higher Cu levels in hepatopancreas tissue are expected since Cu is the active metal moiety of the respiratory transport pigment (hemocyanin) present in crustaceans (Prosser, 1973). Higher Fe levels are likewise expected in liver tissue because of the blood pigment hemoglobin present in fish. The high levels of Ni in shrimp hepatopancreas could provide a means of detecting changes in the concentration of biologically available Ni in the environment.

The second approach was to order the six groups according to the sum total of the seven metals in each one. Using this second criteria the order remained unchanged except that hepatopancreas and liver reversed positions. Zooplankton had the highest total metal content (4.05 mg/g) with livers second (1.05 mg) and hepatopancreas third (0.7 mg). All three of these sample types had total metal contents greater than that in 1976 *Spondylus* samples (*i.e.* 0.42 mg). Gill tissue had a mean total of 0.35 mg with shrimp and fish muscle both having < 0.1 mg.

As discussed previously (Presley and Boothe, *In* Groover, 1977) comparisons between 1975 and 1976 trace metals data were limited due to differences in analytical techniques. However, for 1976 and 1977 essentially all aspects of the sampling regime and analytical techniques were the same. The only major change was the reduction in the number of species analyzed in 1977. Still the species emphasized in 1977 were those that were also analyzed most frequently during 1976. Thus the two data sets are very comparable.

The trace metal levels observed within the major sample groups in 1976 and 1977 were very similar (Table 6.5). The 1976 and 1977 mean values were extremely close for both the fish muscle and shrimp muscle groups. The other groups exhibited some differences between years but none were significant, and no consistent trends were apparent. Such differences were probably just a reflection of the relatively high variability within most groups. The higher mean Pb level in fish livers in 1977 was caused by the higher Pb levels in *Stenotomus caprinus* liver samples which were included in the 1977 group average. More detailed geographic and seasonal comparisons of trace metals data within each sample group are discussed in separate sections below.

Variability in this data set is the key factor which determines how small a change or difference in organismal trace metal concentrations could be detected during a future monitoring program. Relative variability among the six sample groups in the 1977 data set (Table 6.5) was quantitatively characterized in two ways. First, the mean percent coefficient of variation [mean percent CV = (std. dev/mean) x 100] for each metal from the six groups was calculated. Zinc had the lowest variability (mean percent CV = 47). The remaining elements in order of increasing variability were Ni, Cr, Cd, Cu, Fe and Pb (mean percent CV = 60, 69, 72, 77, 85, 113 respectively). As in 1976, Zn had the lowest variability among the three metabolically important elements analyzed (*i.e.*, Cu, Fe. Zn). This fact suggested that Zn metabolism in the organisms sampled may be more independent of environmental and organismal factors than Cu and Fe metabolism. Copper and Fe are directly involved in respiration, a physiological process sensitive to environmental change. Of the elements potentially released into the coastal environment by offshore petroleum-related activities, Ni, Cr and Cd would be the best choices for any monitoring program because of their comparatively low variability. Lead, which also exhibited the highest variability in 1976, should not be included in such an effort.

The second way of examining the relative variability in trace metals content (Table 6.5) was to calculate the mean percent CV for each of the six major sample groups. Zooplankton showed the greatest variability in trace metal concentrations (mean percent CV = 124) with fish liver samples second (mean percent CV = 109). The remaining groups in order of decreasing variability were shrimp hepatopancreas, shrimp muscle, fish muscle and fish gills (mean percent CV = 66, 56, 43, 41, respectively). These values are generally similar to comparable mean percent CV's calculated from the 1976 data set. The value for zooplankton and fish liver samples in 1976 were 128 and 105, respectively. For shrimp muscle the value was 77. However, the mean percent CV for 1977 fish muscle (43) was about half that of the 1976 group (84). This reduction was probably a reflection of the fact that only six fish species were analyzed in 1977 compared to 20 in 1976. The 1977 mean percent CV for gill tissue (41) was similarly much lower than the 1976 value (84). A reduction in the number of sampling periods for these samples in 1977 compared to 1976 (*i.e.*, three versus nine, respectively) was at least partially responsible for this observed decrease in variability.

The amount of variability in trace metal concentrations within several of the groups in Table 6.5 was high. Probable reasons for this variability were discussed previously (Presley and Boothe, *In* Groover, 1977). For zooplankton, these included the variable species composition of the samples and the variable incorporation of aluminosilicate detritus by zooplankton over space and time. Possible reasons for other groups were the lumping of numerous different species in a single group, the calculation of means and variances using less-than values and small differences in sample preparation techniques. Because of the lack of replicate analyses in the 1975 and 1976 studies, however, it was difficult to evaluate how much of the observed variability was due to the above factors and how much was simply that naturally found among similar individuals of the same species from any one place and time.

This distinction is an important one. The amount of intra-station variability in trace metal levels among similar individuals of a single species should approach the minimum variability observable under practical conditions. The majority of controllable sources of variability (*e.g.*, different sampling equipment or techniques, different location or time, different species populations, etc.) are minimized. Thus the magnitude of this intra-station variability will determine how small a difference in trace metal concentrations between stations can be detected statistically under optimal conditions. Such estimates are essential in planning the sampling strategy for any monitoring effort.

The goal of the 1977 study was to obtain estimates of intra-station variability in trace metal levels by analyzing liver and hepatopancreas tissue from similar individuals of the same species collected at the same time and place. For fish liver samples, the mean percent CV of such replicate samples for all seven metals was approximately 45 compared to 109 for the fish liver group as a whole (Table 6.5). The comparable mean percent CV for replicate hepatopancreas samples was also about 45 compared to 66 for the whole group. Intra-station variability was significantly less than that observed among groups of stations. This situation was encouraging because it suggested that reasonable differences could be detected between stations. This general level of variability (*i.e.* mean percent CV ≃ 45) indicated that with a sample size of five replicate analyses, a 50% difference between the means of two samples could be detected statistically ($p < 0.05$; Sokal and Rohlf, 1969).

Twelve zooplankton samples were run in triplicate during 1977. The mean percent CV of these twelve sets of replicate analyses for all seven metals was 19 compared with 124 for the zooplankton group as a whole (Table 6.5). This significant reduction in variability suggested that much of the variability observed in the zooplankton group was caused by grouping dissimilar samples and was not due to extreme intrinsic variability among similar, replicate samples. This level of intra-station variability indicated that a 40% difference between sample means could be detected statistically at $p < 0.05$ with a sample size of five.

Zooplankton

Geographic trends observed in the 1977 zooplankton data were similar to those observed in 1976. Cadmium exhibited a consistent, definite increase in concentration with increasing distance from shore. A one-way analysis of variance test (Sokal and Rohlf, 1969) showed that mean Cd concentrations at Stations 1, 2 and 3 of all transects were significantly different at $p << 0.01$. This trend has been observed in each of the three years of the STOCS study. The increase in Cd correlated with the decrease in zooplankton biomass observed offshore (see Dr. Park's zooplankton report Chapter 14). Lead concentrations in zooplankton generally decreased away from shore although the differences were not significant due to considerable variability in the data. This trend was also observed in 1976 and was probably a result of offshore samples being farther away from coastal sources of Pb input to the STOCS area.

The concentrations of all metals except Cd were highest in Fall season zooplankton samples. However, this difference was unknown but it could be related to differential incorporation of aluminosilicate detritus among the seasons. Seasonal trends in zooplankton trace metal levels were not observed in 1976.

As expected the concentration of suspended matter was significantly greater at inshore stations. The trace metal levels observed in the particulate matter samples were extremely variable. No consistent relationship was observed between the concentrations of metals in suspended matter and the levels found in zooplankton sampled concurrently. These analyses did demonstrate that significant amounts of the trace metals present in the particulate matter were released into solution during the digestion procedure used to prepare organism samples.

Fish Liver

Table 6.6 gives the mean concentrations of trace metals in fish liver tissue from five fish species. The values in Table 6.6 are annual means. All samples of each species collected and analyzed during 1977 were used in calculating these values. In the case of MNK samples (*L. campechanus* and *R. aurorubens*), both pooled and individual liver tissue sample data were included in the means. Only liver tissue samples from these two

TABLE 6.6

AVERAGE CONCENTRATIONS OF TRACE METALS IN FISH LIVER TISSUE IN 1976 AND 1977

Species	Number of Samples	Year Collected	Concentration (ppm dry weight ± 1 standard deviation)					
			Cd	Cr	Cu	Fe	Ni	Pb
Lutjanus campechanus	22	1977	1.6 ± 0.8	0.04 ± 0.04	17 ± 8	600 ± 300	0.2 ± 0.1	0.2 ± 0.1
	16	1976	1.9 ± 0.8	0.05 ± 0.02	20 ± 15	500 ± 300	0.1 ± 0.07	0.2 ± 0.1
Pristipomoides aquilonaris	39	1977	3.9 ± 3.2	0.08 ± 0.08	25 ± 18	600 ± 300	<0.3 ± 0.2	0.2 ± 0.1
Rhomboplites aurorubens	24	1977	12 ± 6	0.08 ± 0.08	30 ± 18	1300 ± 700	0.3 ± 0.2	0.3 ± 0.2
	12	1976	14 ± 9	0.1 ± 0.06	30 ± 30	1300 ± 800	0.4 ± 0.3	0.6 ± 0.4
Serranus atrobranchus	3	1977	1.3 ± 0.2	<0.03 ± 0.03	4 ± 1	200 ± 60	<0.6 ± 0.1	0.1 ± 0.1
Stenotomus caprinus	27	1977	7 ± 6	0.2 ± 0.07	45 ± 28	1000 ± 600	1.5 ± 1.1	6 ± 5

TABLE 6.6 CONT.'D

Species	Number of Samples	Year Collected	Concentration (ppm dry weight ± 1 standard deviation)			
			Zn	V	Al	Ca
Lutjanus campechanus	22	1977	120 ± 30	0.35 ± 0.25	25 ± 15	750 ± 450
	16	1976	120 ± 40	0.65 ± 0.50	70 ± 90	9,000 ± 18,000
Pristipomoides aquilonaris	39	1977	120 ± 40	0.75 ± 0.70	40 ± 25	700 ± 650
Rhomboplites aurorubens	24	1977	360 ± 180	1.7 ± 1.4	45 ± 110	1,000 ± 600
	12	1976	600 ± 400	3.5 ± 2.5	40 ± 20	750 ± 30
Serranus atrobranchus	3	1977	60 ± 4	0.70 ± 0.55	80 ± 11	1,300 ± 300
Stenotomus caprinus	27	1977	200 ± 80	2.5 ± 2.0	55 ± 30	1,200 ± 600

species were analyzed in 1976. The means for both years were very similar. The few differences observed were relatively small and none are significant.

The average total amount of the seven trace metals in the liver tissue of each species was determined. The five species fell into three groups on the basis of this parameter. Livers of *Rhomboplites aurorubens* (1.7 mg/g dry weight) and *Stenotomus caprinus* (1.45 mg/g) contained significantly more of these metals than the other species. Comparatively high levels of Cd, Fe and Pb (in the case of *Stenotomus*) were responsible for this difference. Because of the higher levels, these two species would be logical choices for any future monitoring program.

Livers of *Lutjanus campechanus* (0.74 mg/g) and *Pristipomoides aquilonaris* (0.75 mg/g) contained an intermediate amount of the seven metals. *Serranus atrobranchus* liver tissue contained the smallest amount of metals of any species (0.27 mg/g). Because the livers of this species were so small and difficult to exise only one set of three replicate samples was analyzed. Had more liver samples been analyzed, the average amount of metals observed might have been higher.

There appeared to be few significant correlations between liver trace metal concentration and fish length or total liver dry weight. The metabolically important metals (Cu, Fe and Zn) exhibited the best relationships, but few were significant because of scatter in the data. Zinc did show a significant positive correlation with fish length for *Stenotomus caprinus*. Iron showed a negative correlation for the same species.

All samples of *Pristipomoides* and *Stenotomus* were collected at offshore stations, primarily Station 3 on all four transects. This situation provided an excellent opportunity to observe any north-south trends in liver trace metal levels. *Stenotomus* samples from Transects I and II exhibited generally higher levels of Cd, Ni and Pb than comparable samples from Transects III and IV. Some of the differences were significant. *Pristipomoides* exhibited a similar trend for Cd, but the relationship was weaker for Ni and Pb.

Annual mean trace metal levels in MNK liver tissue samples (both pooled and individual) according to collection site are given in Table 6.7. The data used in this table were the same as that in Table 6.6. No significant or consistent differences between the 1976 and 1977 data were observed. Trace metal levels in *Rhomboplites* were generally not significantly different from concentrations in the same tissues from *Lutjanus*. At both bank stations, however, Cd levels in *Rhomboplites* were significantly greater than those in *Lutjanus*. Also in a majority of comparisons (21/28) at both stations, the metal levels in *Rhomboplites* were higher than comparable ones in *Lutjanus*. This relationships was also observed in 1976. Within each species, the concentrations of all metals studied were similar for both Hospital Rock and Southern Bank. Too few samples were analyzed in 1977 to distinguish any possible seasonal trends in trace metal levels. No seasonal trends were observed in 1976.

Fish Gills

Table 6.7 gives the annual mean trace metal concentrations on MNK pooled gill samples collected at Hospital Rock and Southern Bank. Only two gill samples of each MNK species (*Lutjanus* and *Rhomboplites*) were analyzed from each bank station during 1977. Although the trace metal content of gill tissue was generally less than that of liver tissue from the same species, the relationships were very similar to those observed for liver samples. No consistent or significant difference between the

TABLE 6.7

AVERAGE CONCENTRATIONS OF TRACE METALS IN MACRONEKTON GILL AND LIVER TISSUE IN 1976 AND 1977

Species	Bank Station[1]	No. of Samples	Tissue[2]	Year	Concentration (ppm dry weight ± 1 standard deviation				
					Cd	Cr	Cu	Fe	Ni
Lutjanus campechanus	HR	2	G	77	0.14 ± 0.08	0.1 ± 0.1	1.8 ± 0.9	200 ± 60	0.6 ± 0.1
		6		76	0.16 ± 0.14	0.1 ± 0.06	1.8 ± 0.8	100 ± 40	0.5 ± 0.2
	SB	2	G	77	0.2 ± 0.04	0.1 ± 0.04	1.8 ± 0.1	230 ± 100	0.6 ± 0.3
		6		76	0.5 ± 0.4	0.1 ± 0.07	1.5 ± 0.6	110 ± 30	0.4 ± 0.2
Rhomboplites aurorubens	HR	2	G	77	0.6 ± 0.1	0.1 ± 0.01	3.0 ± 0.0	350 ± 70	0.6 ± 0.0
		3		76	0.6 ± 0.1	0.2 ± 0.1	2.7 ± 1	240 ± 110	0.6 ± 0.1
	SB	2	G	77	0.4 ± 0.2	0.1 ± 0.01	3.0 ± 0.0	240 ± 80	0.6 ± 0.0
		7		76	2.1 ± 1.8	0.1 ± 0.07	4.3 ± 4	160 ± 80	0.4 ± 0.1
Lutjanus campechanus	HR	12	L	77	1.2 ± 0.4	0.04 ± 0.05	14 ± 4	540 ± 180	<0.1 ± 0.0
		6		76	1.5 ± 0.6	0.05 ± 0.01	22 ± 23	680 ± 300	0.1 ± 0.0
	SB	10	L	77	2.0 ± 1.0	0.04 ± 0.03	20 ± 10	610 ± 460	<0.2 ± 0.2
		6		76	1.6 ± 0.5	0.06 ± .02	13 ± 6	350 ± 150	0.1 ± 0.0
Rhomboplites aurorubens	HR	11	L	77	8 ± 2	0.03 ± 0.02	25 ± 23	800 ± 280	<0.2 ± 0.1
		3		76	15 ± 4	0.1 ± 0.08	14 ± 4	1900 ± 1100	0.7 ± 0.4
	SB	13	L	77	14 ± 7	0.12 ± 0.09	35 ± 11	1700 ± 670	<0.4 ± 0.2
		5		76	9 ± 6	0.08 ± 0.03	30 ± 50	1100 ± 600	0.2 ± 0.0

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[1] HR = Hospital Rock; SB = Southern Bank

[2] G = gill tissue; L = liver tissue (both pooled and individual samples)

TABLE 6.7 CONT.'D

Species	Bank Station[1]	No. of Samples	Tissue[2]	Year	CONCENTRATION (ppm dry weight ± 1 standard deviation)				
					Pb	Zn	V	Al	Ca
Lutjanus campechanus	HR	2	G	77	0.3 ± 0.1	75 ± 7	0.16 ± 0.06	55 ± 14	65,000 ± 30,000
		6		76	1.0 ± 1.7	70 ± 23	0.50 ± 0.20	95 ± 100	90,000 ± 40,000
	SB	2	G	77	0.3 ± 0.0	85 ± 7	0.20 ± 0.07	55 ± 0	75,000 ± 35,000
		6		76	0.5 ± 0.3	70 ± 30	0.60 ± 0.20	110 ± 100	110,000 ± 19,000
Rhomboplites aurorubens	HR	2	G	77	1.7 ± 0.1	85 ± 7	1.3 ± 0.14	65 ± 0	21,000
		3		76	3.3 ± 3.9	160 ± 140	6	220	13,000
	SB	2	G	77	1.3 ± 0.1	95 ± 7	1.1 ± 0.80	55 ± 40	75,000
		7		76	1.6 ± 1.4	75 ± 8	2.0 ± 1.0	75 ± 60	65,000 ± 45,000
Lutjanus campechanus	HR	12	L	77	0.2 ± 0.07	110 ± 18	0.25 ± 0.14	20 ± 5	900 ± 500
		6		76	0.2 ± 0.08	130 ± 50	0.90 ± 0.70	120 ± 130	25,000 ± 30,000
	SB	10	L	77	0.2 ± 0.1	120 ± 39	0.45 ± 0.30	30 ± 20	500 ± 300
		6		76	0.2 ± 0.1	110 ± 30	0.40 ± 0.10	30 ± 5.0	60,000 ± 50,000
Rhomboplites aurorubens	HR	11	L	77	0.3 ± 0.2	310 ± 220	1.2 ± 0.65	20 ± 5	1,000 ± 650
		3		76	0.8 ± 0.5	490 ± 350	4.0 ± 1.0	65 ± 25	130,000 ± 180,000
	SB	13	L	77	0.4 ± 0.2	400 ± 140	2.0 ± 1.8	70 ± 150	1,000 ± 500
		5		76	0.4 ± 0.2	340 ± 390	1.0 ± 0.50	30 ± 20	50,000 ± 70,000

1976 and 1977 data was observed. As seen with liver samples, trace metal concentrations in *Rhomboplites* gills were consistently higher (24/28 comparisons) than those of *Lutjanus*, but these differences were generally not significant. One exception was Pb which was significantly higher in the gill tissue of *Rhomboplites* at both bank stations. This relationship was also observed in 1976 but was not significant due to variability in the data. Within each species, the concentrations of all metals studied was similar for both sampling sites. No seasonal trends were apparent from the limited data. No such trends were observed in 1976.

Shrimp Hepatopancreas

This shrimp tissue was first analyzed in 1977. Trace metal levels were similar in the hepatopancreas tissue of both penaeid shrimps analyzed. As with fish livers, few significant correlations were observed between trace metal levels and length of shrimp or total hepatopancreas dry weight. A significant positive correlation existed between Zn concentration and total length. A negative correlation was observed between Fe and length.

Seasonal differences were observed but none were significant due to variability in this grouped data. Cadmium and Ni concentrations were maximum during the winter seasons. Significant differences between levels of certain trace metals at different stations were observed, but no real geographic trend was apparent. The concentrations of Ni and Pb were generally highest at Transect II stations.

The relatively high concentrations of Cd, Ni and V accumulated in hepatopancreas tissue make this sample type a logical choice in any monitoring effort. In addition, shrimp are both ecologically and economically important in the STOCS area. The comparatively low intra-station variability of these elements should make it possible to detect differences between stations with only a reasonable number of replicate analyses (*i.e.* 5-10).

Fish Muscle

Table 6.8 gives the annual mean concentrations of trace metals in muscle tissue from the six species of fish analyzed. The levels observed in 1977 were very similar to those found in 1976. The trace metal concentrations in fish muscle were generally low and uniform among the different species. Even macronekton samples collected at the two bank stations had trace metal levels similar to fish collected from the 12 transect stations. The only significant difference was also observed in 1976. No significant geographical or seasonal trends in trace metal levels in fish muscle were observed for any of the species.

Fish muscle would not be a useful sample type for any future monitoring program in the STOCS area. The trace metals of real interest in such a program (*i.e.* Cd, Cr. Ni, Pb, V) all occur at levels in fish muscle that are near or below the detection limits of even flameless AAS. With so many less-than values, no real estimate of intra-station variability in trace metal levels can be obtained. As a result, statistical detection of differences between samples is generally not possible.

In addition, fish muscle was not a good indicator of elevated trace metal levels in other tissues in the same fish. For example, *Stenotomus caprinus* livers contained significantly higher levels of Pb than did the livers of other species. Still the levels of Pb in *Stenotomus* muscle were the same as other species. More sensitive and similarly rapid techniques for routine determination of such low elemental concentrations in biological materials were not available.

TABLE 6.8

AVERAGE CONCENTRATIONS OF TRACE METALS IN POOLED SAMPLES OF FISH MUSCLE IN 1976 AND 1977

Species	Number of Samples	Year Collected	Concentration (ppm dry weight ± standard deviation)				
			Cd	Cr	Cu	Fe	Ni
Lutjanus campechanus	4	1977	<0.01 ± 0.00	<0.02 ± 0.01	0.7 ± 0.1	3.2 ± 0.5	<0.06 ± 0.01
	17	1976	0.03 ± 0.00	0.03 ± 0.01	0.8 ± 0.3	5.4 ± 2.8	0.06 ± 0.02
Pristipomoides aquilonaris	14	1977	<0.01 ± 0.00	0.04 ± 0.01	1.2 ± 0.6	4.2 ± 1.6	<0.08 ± 0.03
	28	1976	0.02 ± 0.02	0.04 ± 0.04	1.3 ± 0.5	3.8 ± 1.8	0.08 ± 0.03
Rhomboplites aurorubens	4	1977	<0.01 ± 0.00	<0.02 ± 0.01	0.6 ± 0.1	5.8 ± 0.5	<0.07 ± 0.00
	14	1976	0.01 ± 0.01	0.03 ± 0.03	1.0 ± 0.2	6.9 ± 2.7	0.05 ± 0.02
Serranus atrobranchus	11	1977	<0.01 ± 0.00	<0.06 ± 0.01	0.9 ± 0.5	4.1 ± 1.1	<0.08 ± 0.01
	11	1976	0.02 ± 0.01	0.03 ± 0.01	0.8 ± 0.3	2.9 ± 1.5	0.08 ± 0.02
Stenotomus caprinus	11	1977	<0.01 ± 0.00	0.04 ± 0.01	1.0 ± 0.3	4.9 ± 0.9	<0.07 ± 0.02
	9	1976	0.02 ± 0.01	0.03 ± 0.01	0.9 ± 0.2	4.6 ± 1.9	0.10 ± 0.03
Trachurus lathami	8	1977	0.01 ± 0.00	0.06 ± 0.03	2.5 ± 0.7	18 ± 5.6	0.09 ± 0.01
	12	1976	0.05 ± 0.03	0.04 ± 0.05	2.1 ± 0.7	13 ± 6.1	0.13 ± 0.10

TABLE 6.8 CONT.'D

Species	Number of Samples	Year Collected	Concentration (ppm dry weight ± standard deviation)				
			Pb	Zn	V	Al	Ca
Lutjanus campechanus	4	1977	<0.03 ± 0.01	10 ± 0.8	< 0.05 ± 0.01	15 ± 3	400 ± 250
	17	1976	0.03 ± 0.01	12 ± 10	0.20 ± 0.10	30 ± 25	4,500 ± 7,000
Pristipomoides aquilonaris	14	1977	<0.05 ± 0.02	10 ± 4.2	<0.06 ± 0.02	18 ± 2.5	600 ± 300
	28	1976	0.04 ± 0.02	8.2 ± 4.8	0.20 ± 0.10	30 ± 10	1,600 ± 3,000
Rhomboplites aurorubens	4	1977	<0.04 ± 0.02	9.5 ± 1.0	<0.06 ± 0.01	14 ± 0.7	400 ± 70
	14	1976	0.03 ± 0.02	11 ± 6.3	0.20 ± 0.10	20 ± 7.0	6,500 ± 13,000
Serranus atrobranchus	11	1977	<0.03 ± 0.01	11 ± 0.8	0.08 ± 0.02	19 ± 6.5	2,000 ± 1,300
	11	1976	0.04 ± 0.04	8.5 ± 3.8	0.20 ± 0.10	30 ± 7.0	1,000 ± 300
Stenotomus caprinus	11	1977	<0.05 ± 0.01	12 ± 1.2	<0.06 ± 0.02	15 ± 3.0	550 ± 250
	9	1976	0.05 ± 0.04	11 ± 3.7	0.20 ± 0.10	30 ± 13	800 ± 500
Trachurus lathami	8	1977	<0.05 ± 0.02	17 ± 4.8	<0.10 ± 0.06	30 ± 16	1,200 ± 800
	12	1976	0.07 ± 0.04	21 ± 4.1	0.10 ± 0.10	20 ± 8.0	700 ± 250

Shrimp Muscle

Trace metal levels in shrimp muscle are given in Table 6.5. The levels in *Penaeus aztecus* and *Penaeus setiferus* were very similar. No separate tabulation by species was necessary. The levels of all but the metabolically important metals were very low. Concentrations in shrimp muscle in 1977 and 1976 were essentially the same. No seasonal or geographic trends were apparent in the 1977 data. Shrimp muscle would not be a good sample type for any monitoring efforts. The reasons for this recommendation are the same as those discussed above regarding fish muscle.

CONCLUSIONS

The primary purpose of this three year study was to establish basic concentrations of trace metals in marine organisms within the STOCS study area. This characterization data could then be used to determine if future oil and gas exploration and production in the area contributed significant amounts of trace metals to the biota.

No indication of significant heavy metal pollution was observed in the STOCS area. For many of the types of organism samples analyzed, trace metal levels were generally low and quite uniform over the entire region. Adequate initial data in the form of annual mean trace metal concentrations (Tables 6.5 - 6.8) were obtained for certain tissues of several species of organisms during this study. These species and tissues included: *Penaeus aztecus* and *P. setiferus* (muscle and hepatopancreas); *Lutjanus campechanus* and *Rhomboplites aurorubens* (muscle, gill and liver); *Pristipomoides aquilonaris* and *Stenotomus caprinus* (muscle and liver); *Serranus atrobranchus* and *Trachurus lathami* (muscle); *Spondylus americanus* (whole organism).

More detailed trace metals data were obtained during the 1977 study when extensive replicate analyses of liver and hepatopancreas tissue from certain of the above species were conducted.

Intraspecific variability in trace metal levels within the STOCS area as a whole was high. However, data from the 1977 study showed that this variability among individuals of the same species collected at one place and time was much lower for most of the metals studied. Thus in any future monitoring program, emphasis should be placed on replicate analyses of a few sample types from a few key stations. In this way relatively small differences in trace metal levels could be detected between sampling sites. For example, at the level of intraspecific variability observed for most metals, a 50% difference in means between stations could be distinguished statistically ($p < 0.05$) on the basis of 5-10 replicate analyses of the same sample type at each station. The large amount of general data obtained from this three year study could be used to select the best monitoring sites for such a program.

Of the sample types analyzed during this study, three are best suited for any future monitoring effort in the STOCS region. *Spondylus americanus* (spiny oyster) would be the best choice. This species is a sedentary, filter-feeding bivalve. It contains measurable levels of all the metals studied and the intra-station variability in metal levels is quite low. *Spondylus*, however, has a very discontinuous distribution within the STOCS area and could be difficult to obtain in suitable numbers. Thus the use of this organism could cause problems depending on the location of the area to be monitored. A solution to this limitation would be to transport individual *Spondylus* to the areas of interest and allow them to "sample" the ambient metal levels for several weeks or months.

The remaining two sample types are fish liver (*i.e. L. campechanus, P. aquilonaris, R. aurorubens, S. caprinus*) and shrimp hepatopancreas (*P. setiferus, P. aztecus*). Although relatively mobile, these species are widely distributed within the STOCS area and generally available in sufficient numbers most of the year. These tissues contain measurable amounts of most of the metals studied and their intra-station variability in trace metal levels is very acceptable.

LITERATURE CITED

Anonymous. 1971. Instruction manual for Jarrell-Ash Model 810 atomic absorption spectrophotometer (Engineering Pub. #82-810). Jarrell-Ash Division, Fisher Scientific Co., Waltham, Mass. 54pp.

________. 1972. Atomic absorption analytical methods. Jarrell-Ash Division, Fisher Scientific Co., Waltham, Mass.

________. 1974. Analytical methods for atomic absorption spectroscopy using the HGA-2100 graphite furnace (#990-9972). Perkin-Elmer Corp., Norwalk, Conn. 43pp.

Cotton, F. A. and G. Wilkinson. 1972. Advanced inorganic chemistry. Interscience Publishers, New York.

Girardi, F. and E. Sabbioni. 1968. Selective removal of radiosodium from neutron-activated materials by retention on hydrated antimony pentoxide. J. Radio. and Chem. 1:169-178.

Presley, B. J. and P. N. Boothe. 1977. Trace Metals Project. pp 18-1 - 18-49. *In* R. D. Groover (ed.) Environmental studies, South Texas Outer Continental Shelf, Biology and Chemistry. 1976 Final Report to the Bureau of Land Management, Washington, D. C. Contract No. AA550-CT6-17.

Prosser, C. L. 1973. Comparative animal physiology. 2nd Edition. W. B. Saunders, Philadelphia.

Reed, J. H. 1977. Barium and vanadium determination by neutron activation analysis - a study of procedures. Report submitted to the Bureau of Land Management, Washington, D. C. (Contract AA550-CT7-38) by Science Applications Inc, La Jolla, California, 84pp.

Schlueter, D. J. 1972. HEVESY, a general activation analysis computer program. Report TEES. 9002-1972-1. Texas A&M University, College Station, Texas. 41pp.

CHAPTER SEVEN

SEDIMENT TEXTURE

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ABSTRACT

During 1977, 576 samples representing 6 replicates at each of 25 line and four bank stations sampled during each of 3 seasons (winter, spring, and fall) and 18 miscellaneous samples per season taken for mycological and bacteriological studies were analysed for sediment texture with results reported as mean grain size, standard deviation, skewness, kurtosis (moment parameters) and percentages of sand, silt, clay, and fine clay. The purpose of these data was to provide substrate textural data for benthic organism studies. The samples also provided analyses of textural variability which generally increased from a low at the outer shelf edge to a maximum just seaward of the boundary between shoreface sands and offshore muddy bottoms and then decreased again as sand became dominant shoreward. Five stations are interpreted as having significant seasonal changes. The most consistent pattern seems to have been a general coarsening in muddy sand bottoms of the inner shelf especially between winter and spring sampling. This supports a suggestion of this trend observed in 1976; but a similar trend on the outermost shelf observed in 1976 was poorly supported.

INTRODUCTION

Purpose

The purpose of this study was to provide sediment grain size distribution (texture) data for comparison with the results of biological and chemical analyses of bottom samples, especially analyses for macroinfauna and microbiology. The replication of samples at a station and through time allowed analysis of the variance of sediment texture within sampling stations and analysis of seasonal trends in grain size characteristics.

MATERIALS AND METHODS

Sediment texture samples were taken from Smith-McIntyre grab samples. Each sediment texture sample consisted of not less than 100 g sediment.

Textural analyses were done by the rapid sediment analyer (Schlee, 1966) for the sand-sized fraction and by the pipette method (Folk, 1974) for the mud fraction. The sample was homogenized by kneading in the plastic sample bag. Twenty (20) cc were extracted, dispersed in hydrogen peroxide, diluted to about 0.5 ℓ and allowed to stand for two to three days. The clearer supernate was decanted through a 1.2 μm MILLIPORE filter and the filtered sediment was returned to the beaker. The sediment was resuspended and poured through a 0.062 mm screen. The screen (preweighed) and trapped sand were dried, weighed, and set aside for later settling tube analysis.

The mud fraction was transferred to a graduated cylinder and the sample was diluted to 1 ℓ, stirred, and allowed to stand overnight. If no flocculation occurred (none ever did), the temperature was measured, settling rates were calculated by Stoke's Law, and withdrawal times and depths were calculated to obtain nine intervals from 4 to 10 phi. Twenty (20) ml samples were pipetted at the appropriate times, transferred to preweighed beakers, oven dried, weighed, and size fraction weights calculated.

A representative portion of the sand fraction was introduced into a settling tube and fall times were recorded continuously. Graphs were read for the proportion of sand at each 0.25 phi interval. These data were used to compute moment and graphic grain size parameters by standard methods (McBride, 1971).

Of particular concern for data comparison purposes was the handling of data for the fine clay fraction. The last pipette measurement defines the quantity of material finer than 10 phi. Extrapolation of the cumulative curve beyond 10 phi very commonly indicates that all of the sediment is coarser than 14 phi. Therefore, a common computation procedure is to extrapolate cumulative curves to 100% at 14 phi (Folk, 1974). One set of grain size distribution parameters was done on this basis.

The Coulter Counter technique defines the relative abundances of material in each of several size classes over the range of measurement. However, it does not determine how much of the mud fraction is within that range of measurement and how much is finer than the lower limit of the smallest class measured (10.6 phi). Therefore, the computational procedure assumes all of the mud is coarser than the finest size measured. This, in effect, redistributes the fine clays over all the silt and clay classes, and therefore, computes coarser mean sizes. To determine the extent of this and other possible effects, the raw data were used to compute grain size parameters by the technique necessitated by the Coulter Counter analysis technique.

RESULTS

Tabulation

The mean grain size, standard deviation, skewness, and kurtosis determined by the method of moments and percentages of sand, silt, clay and clay finer than 10 phi for each of the 576 grab samples are presented in Table 1, Appendix F. Means and standard deviations of each of these values for the groups of six replicates taken seasonally at each station are also presented in this table.

The statistical parameter of standard deviation is used in two contexts in this report. The range of grain sizes determined by the analysis of a single sample is the standard deviation of that sample. It is expressed in phi (0) units and is abbreviated s.d.. This is a measure of the degree of sorting of grains in that sample and is usually referred to as sorting. Variability within a group of samples (*e.g.*, the six replicates at a particular station) is also expressed as standard deviation of some grain size distribution parameter (usually mean grain size) measured on each sample in the group. This parameter is abbreviated s, and is referred to as variability or variance (variance is mathematically defined as s^2).

Station Variability

The lowest variability between replicate grab samples within a station was for the clayey, outer shelf Stations 3/I, 6/I, 3/II, 5/II, 6/II, 2/III, 3/III, 5/III, 6/III and 7/IV. These stations generally displayed mean grain sizes well within the clay range (8.5 to 9.6 0) and averaged only 5% sand and 33% silt. Station 6/I was somewhat coarser (7.7 0) with 18.5% sand and 37% silt, but it had the same low variability between grab samples. The standard deviation (s) between mean grain sizes of replicate grab samples was less than 0.32 0 for these stations. Station 5/II was an exception to this because of a high s (0.80 Ø) for the spring cruise. However, LORAC navigation failed on Transect II during the spring cruise, so this value probably represented a different station than sampled on the other seasonal cruises. Unusually high s values for Stations 4/II and 6/II suggested that the same navigational variance affected these stations. Equally low variability was characteristic of Station 4/III which displayed a slightly muddy (10%), very fine sand on the innermost shelf.

Almost all stations with low replicate variability were also characterized by relatively low standard deviations of grain sizes within each analyzed sample. The values were generally below 3 Ø. The coarsest of the outer shelf stations, 6/I, was the exception to this with a s of 3.5 Ø.

Four stations within the low variability group (3/I, 3/II, 2/III and 3/III) were tested for intragrab sample variance by analyzing four or five replicates from each of three grab samples from each station. These analyses showed that except for Station 2/III there was no significant difference between grab samples. This implied that the best textural values for any grab sample from these stations was usually obtained by averaging all analyses for that station.

Station 5/I was unique in that it was as fine textured (9.5 Ø) and well sorted (s = 2.90 Ø) as the other outer shelf stations, but variation between grab samples was consistently high. Sandiness was particularly variable with many samples having less than 1% but some having over 10%.

The bank stations HR 2, HR 4, SB 2, and SB 4 were within the outer shelf province of clayey sediments and generally had mean grain sizes within the range of 8-10 Ø. However, bottom conditions were extremely variable at these sites, and variability between replicate samples ranged from very low (s = 0.05 Ø) to very high (s = 1.2 Ø) for any particular sampling period.

Station 2/II, although coarser than the outer shelf group, had equally low intra-station variability (s = 0.27 0). However, when the spring cruise data point was removed, s became 0.35 0 and this station was more like the group of stations with intermediate variability. This group included Stations 1/I, 2/I, 4/I, 4/II, 1/IV, 2/IV, 4/IV, and 6/IV. Standard deviations for means of groups of replicates (s) for this group varied from 0.35 to 0.55 0. Although the means for these stations were generally in the silt range, silt was almost never predominant, and relative amounts of sand, silt, and clay were extremely variable with each ranging from 20 to 40%. Consequently, sorting was poor (s = 3.5 ± 0.3 0). Three of four stations tested for intragrab sample variance showed that variability within grab samples was as great as between grabs and this accounted for variability between them just as with the low variability group. Station 1/I was the exception to this rule.

Of the five stations with highest variability between replicate grab samples (s = 0.6 to 0.9 0), two (3/IV and 5/IV) were within the Rio Grande delta, two were innermost shelf muds (1/II and 1/III), and one was the uniquely variable Station 5/I. The Rio Grande delta stations had the poorest sorting (s > 4 0) because of the greatest variety of sediment sources. Sorting of the inner shelf muds was equivalent to that of the intermediately variable, mid shelf muds; Station 5/I was clayey with typically good sorting (s = 2.9 0). Two out of the three stations in this group that were checked for intragrab sample variance yielded results that indicated that individual grab samples for this group should be considered to have unique textures as a rule. At Station 1/III variance was primarily due to variability within grab samples.

Seasonal Variability

Absolute seasonal variations fell into two discrete categories. Outer, clayey stations (including two more than in the low variability group, 2/II and 5/I) and innermost, sandy stations (4/I, 4/III and 4/IV). All these had low seasonal variations while all other stations had distinctly higher seasonal variations. However, these variations closely followed intrastation variance, for the most part, and thus could simply reflect that variance.

In order to determine the significance of seasonal variations they were compared to intrastation variations by first ranking all stations by average s values and then by total variance of all 18 grab samples taken during the year at that station. The second measure, in effect, adds seasonal variability to the first. If seasonal variability were due solely to station variability, the two rankings would have been the same; but if real seasonal changes did occur, they would cause a station to drop in ranking relative to stations that had no significant seasonal changes. The ranking comparisons (Table 7.1) showed seven stations that dropped in rank: 1/I, 2/I, 3/I, 3/II, 2/III, 4/III and 2/IV.

A similar comparison was made by plotting the pair of values for each station (Figure 7.1). Again, if seasonal variations were due to intrastation variability, the point for a station would be on or near (nearness measured by one standard deviation of the mean intrastation variance) a line representing equal mean intrastation and total seasonal variance. Although for almost all stations the variance increased when seasonal effects were included, the increases were within one standard deviation of the s value except for Stations 1/I, 2/I, 3/I, 1/II, 3/II, 4/III, 2/IV and 5/IV.

TABLE 7.1

STANDARD DEVIATIONS OF GROUPS OF MEANS

s						total	%
W	S	F	mean	STA	STA	s	change
.13	.07	.11	.10	3/II	6/III	.16	0
.09	.17	.21	.16	6/III	3/II	.20	100
.11	.26	.18	.18	4/III	3/III	.21	11
.15	.16	.26	.19	3/III	7/IV	.26	24
.32	.08	.24	.21	7/IV	6/II*	.27*	13
.14	.50*	.34	.21*	6/II*	4/III	.30	67
.45	.18	.12	.25	3/I	2/II	.30	-
.26	.34	.21	.27	5/III	5/III	.30	11
.31	.20	.30	.27	6/I	6/I	.31	15
.13	.81*	.42	.28*	5/II*	5/II*	.31*	11
.09	.41	.43	.31	2/III	3/I	.32	28
.30	.11*	.39	.35*	2/II	2/III	.34	10
.21	.44	.39	.35	4/IV	4/IV	.36	3
.26	.44	.51	.40	1/I	6/IV	.56	33
.33	.62	.31	.42	6/IV	4/II*	.56	17
.36	.42	.56	.45	2/IV	1/I	.62	55
.53	.82*	.43	.48*	4/II*	5/I	.65	-
.30	1.04	.26	.53	4/I	4/I	.67	26
.35	.72	.56	.54	2/I	1/IV	.67	26
.45	.84	.34	.54	1/IV	3/IV	.71	16
.54	.26	1.02	.61	3/IV	2/I	.72	33
.64	.72	.66	.67	5/I	1/III	.79	16
.54	.73	.78	.68	1/III	5/IV	1.08	44
.69	.68	.89	.75	5/IV	1/II	1.11	25
1.11	.76	.80	.89	1/II	2/IV	1.75	289

W-winter, S-spring, F-fall
First column of stations is ranking by average within station variance; second column of stations is ranking after seasonal effects have been added. See text for further discussion.
* - stations where LORAC navigation was not available; corresponding s values were not included in calculation of mean or total s.

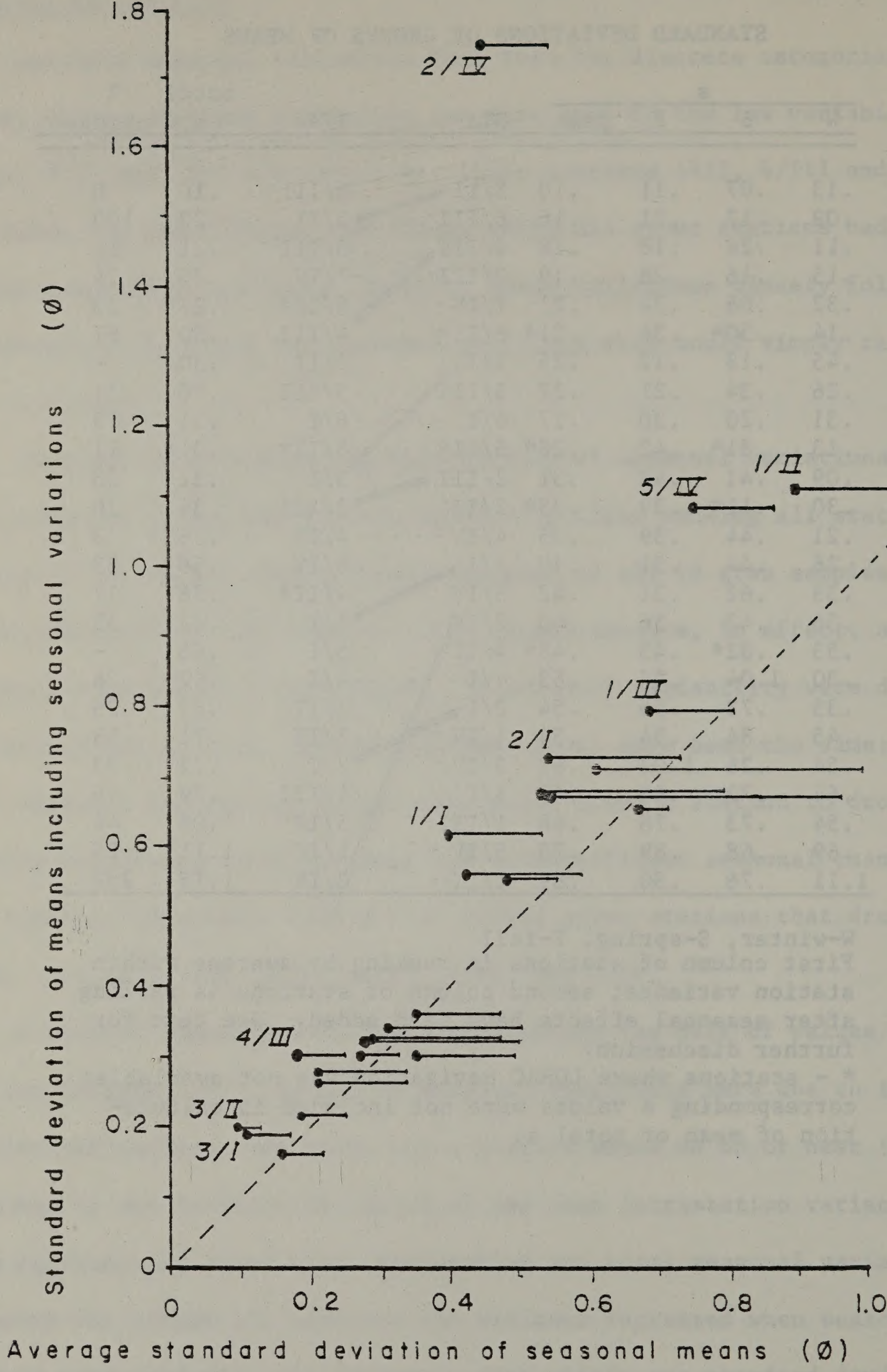

Figure 7.1 Comparison of standard deviations of mean grain size with (ordinate) and without (abscissa) seasonal effects. Horizontal lines are one standard deviation of average of three seasonal values.

Stations can further be compared by noting the percent of increase in varainces of mean grain sizes by adding the seasonal effect. Stations with greatest percent increases were: 1/I, 2/I, 3/I, 3/II, 4/III, 2/IV, 5/IV, and 6/IV (Table 7.1). Stations common to these three estimates of significant seasonal variations were: 1/I, 2/I, 3/I, 3/II, 4/III and 2/IV.

DISCUSSION

Station Variability

Stations varied enough so that each could be treated as having unique characteristics. More efficient or meaningful comparisons with other data could be obtained however, if generalizations could be based on groups or gradients of textural data. The most distinctive group was the outershelf clays. These also graded from finest, best sorted, and least variable texture for the outermost stations (7/IV, 6/III, 3/III and 3/II) to slightly less well sorted, siltier, more variable stations (2/III, 5/III, 5/II, 6/II and 3/I). Station 5/I was very similar to this group but was more variable. Station 6/I was similar in variability but slightly coarser and less well sorted.

Station 4/III was most characteristic of the outer margin (shoreface) of the barrier island sand body where variability was low probably because wave action could constantly maintain a fairly well sorted texture. Slightly seaward of this zone sand usually remained predominant but was mixed with considerable amounts (20-50%) of shelf mud. Stations characteristic of this environment were 1/I, 4/I, 1/IV and 4/IV. The stations on Transect I had fine to very fine sand as the coarse fraction while those from Transect IV had much coarser sand and some gravel in the coarse fraction.

The rest of the stations on Transect IV (3, 4, 5 and 6) were characteristically very poorly sorted, variable mixtures of fine gravel to fine clay. High grab sample variability suggested that bottom conditions were least uniform in this environment with abundant patches of both very clayey and coarse, sandy sediment. Slight navigational errors probably made the greatest differences in bottom conditions sampled in this area.

Maximum variability was characteristic of a zone just seaward of the boundary between shoreface sands and shelf muds. Stations 1/II and 1/III were in this zone. Fine sand was rarely predominant but constituted between 10 and 40% of the sediment and was apparently distributed very heterogeneousl on scales from centimeters to tens of meters. Adequate statistical sampling in this zone and on the Rio Grande delta would require the largest number of replicates, probably more than has been used in the BLM studies.

The last group of Stations (2/I, 2/II and 4/II) represented mid-shelf muds of moderate variability. Although their variability was measurably less than that of the most variable stations, it was generally large enough to require at least as many replicate samples as has been used in the BLM studies of the Texas OCS region (6 or 7).

Seasonal Variability

Although precision navigation was used to relocate stations sampled at different seasons, navigational errors, if they occurred, may have caused the seasonal variations observed. Whether sedimentary processes or navigational errors caused the observed variations could not be determined conclusively, but the specific nature of the seasonal variations, considered in the light of possible physical causes, provided information from which inferences could be drawn.

The greatest seasonal change occurred at Station 2/IV where the winter mean was 5.40 Ø and the sample was about 55% sand. In spring and fall the mean grain size was 8.9 - 9.0 Ø with a sand content of seven to eight percent

A similar, large variation (mean size change of 2.5 0) occurred between the spring sample and the winter and fall samples of the 1976 study. On that occasion LORAC navigation failure accounted for the divergence of the spring sample. Only periodic mud flows and subsequent, irregular winnowing away of almost all fine sediments could account for such drastic changes naturally, and no processes of this nature were known to have operated on the continental shelf in this region. Therefore, it was concluded that the winter sample for Station 2/IV was taken at a different locality than the other seasonal samples.

Four stations (1/II, 2/III, 5/IV and 6/IV) showed significant seasonal changes in sediment texture according to one or two of the methods of estimating significance but not all three. Station 5/IV showed seasonal effects well beyond the range of intrastation variability (Figure 7.1). It did not change rank, only because it was more separated from the next higher ranking station (1/II) by twice as much as any other difference between stations (Table 7.1). In addition, the next higher station had the maximum variability of any station sampled. The change was a coarsening during the spring accomplished by both an increase in sand and a decrease in clay with little change in silt content. This may have occurred from winnowing of clays and some fine silts during the spring when seasonal winds were at a maximum; however, the high spatial variability of the Rio Grande delta and the high probability of at least one navigational error having occurred in this region made navigational variance a slightly more plausible explanation in this case.

Station 6/IV was among the group with a high percentage increase in s with the addition of possible seasonal effects, but no other indicators suggested a real temporal change at this station, and none was believed to be significant.

Station 1/II was similar to 5/IV in its extremely high intrastation variance. Thus the marginal degree to which this station extended beyond one standard deviation of s when seasonal effects were added to intrastation variance, probably did not represent real temporal changes. A further indication of this was that the type of change observed in 1977 was opposite to an equally large seasonal variation in 1976 at this station. Station 2/III dropped in rank in Table 7.1 but showed no other evidence of seasonal change, and none was considered real.

The remaining five stations that showed significant seasonal changes according to all three methods of estimating significance were 1/I, 2/I, 3/I, 3/II, and 4/III. The seasonal changes at Station 3/II followed the most widespread, significant seasonal changes observed in 1976. Those were spring coarsenings at the outer shelf, clayey stations accomplished by reduction in the quantity of finest clays (> 10 0). Stations 6/I, 4/II, 5/II, and 6/II, also followed this trend, but most of these stations lacked the precision LORAC navigation on the spring cruise when coarsening was observed. Furthermore, the spring coarsening was caused by complex variations in sand, silt, and clay contents rather than just loss of fine clays. Many stations (3/I, 3/III, 5/III, 6/III, and 7/IV) in the outer shelf group showed no pattern or opposite seasonal trends. Consequently the trend apparent in the 1976 data of spring coarsening on the outer shelf by winnowing of the finest clays had little support from the 1977 data.

In contrast to the outer shelf stations, the inner shelf stations with high sand contents (30 to 80%) showed similar coarsening trends throughout 1977. Although changes at Station 4/III were relatively small, the small intrastation variance made them significant. Coarsening occurred throughout the year, whereas in 1976 spring coarsening was followed by fall fining at this station. Significant spring coarsening also occurred at Station 1/I.

All coarsenings at inner shelf stations accompanied increases in sand content and decreases in mud content. They may have occurred because of sand deposition, mud erosion, or both. If sand deposition occurred it would imply a general offshore movement of sand from the barrier shoreface. This and mud erosion may have resulted from an increase in wave climate. The coarsening effects apparent in the fall seasonal samples may have been related to such an increase resulting from the passage of a hurricane just south of the study area in August preceding the fall sampling cruises. The effectiveness of this event was supported by some fall coarsening at all inner shelf stations even though Stations 4/I, 1/IV and 4/IV did not pass tests of significance.

Station 2/I behaved similarly to the inner shelf stations during 1977 in that an increase in sandiness caused the spring texture to be significantly coarser than the texture for the winter samples. However, there was no significant change between spring and fall at this station.

Station 3/I showed the opposite trend of spring fining and fall coarsening. These changes apparently resulted from clay deposition in the spring and silt deposition in the fall. These events represented the deeper water equivalents to coarser particle deposition at the inner shelf stations.

CONCLUSIONS

The suite of 25 line stations sampled as a part of the BLM sponsored STOCS study included a wide range of sediment textures from silty clays to muddy sands. Several textural characteristics could be described for the suite in terms of groups of similar stations and gradients of properties.

The textural gradients from outermost shelf landward were as follows. There was a silty (30%) clay of very uniform texture from sample to sample which sometimes showed a seasonal tendency to coarsen by winnowing of finest clays during the early spring at Stations 3/II, 3/III, 6/III, and 7/IV. A slightly coarser, more variable silty clay occurred at Stations 3/I, 5/I, 6/I, 5/II, 6/II, 2/III and 5/III. There was quite a variable sand-silt-clay, midshelf mixture at Stations 2/I, 2/II and 4/II in the northern part of the study area and a similar group with somewhat more variability at least partly because of a much coarser sand mode with some gravel at Stations 2/IV, 3/IV, 5/IV, and 6/IV on the Rio Grande delta. Further landward were the most variable inner shelf sandy muds at Stations 1/II and 1/III. The most landward stations (Stations 1/I, 4/I, 1/IV and 4/IV) had moderately variable muddy sands near the barrier shoreface sand-offshore mud boundary. Finally, Station 4/III was within the shoreface sands where variability became as low as at the outermost stations due to the efficiency of wave action constantly sorting the bottom sediments in shallow water. At the inner shelf stations there was also a suggestion of seasonal coarsening in early spring and a year-long coarsening in 1977 perhaps related to hurricane generated waves between spring and fall sampling.

LITERATURE CITED

Folk, R. L. 1974. Petrology of sedimentary rocks. Austin, Hemphill. 182pp.

McBride, E.F. 1971. Mathematical treatment of size distribution data. pp 109-127. *In* Carver, R. E. (ed), Procedures in sedimentary petrology. New York, Wiley-Interscience.

Schlee, J. 1966. A modified Woods Hole rapid sediment analyzer. Jour. Sed. Petrology 36:403-413.

CHAPTER EIGHT

WATER COLUMN BACTERIOLOGY

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ABSTRACT

The 1977 water column bacteriology studies were intiated as a part of the South Texas Outer Continental Shelf (STOCS) program to identify and to quantitate the heterotrophic and hydrocarbonoclastic microbial populations at Stations 1-3, Transect II; to determine the oil degradation potential of the hydrocarbonoclastic bacteria; to study the ecological succession of mixed bacterial cultures in the presence of oil; and to study the effects of different nutrients on metabolic activities of pure and mixed bacterial cultures.

These studies were conducted on water samples collected on the seasonal and monthly cruises, at a depth of one meter. The results have provided a limited, but preliminary, overall picture of the distribution and abundance of the heterotrophic and hydrocarbonoclastic bacterial genera occurring in the water column at the one meter depth.

Heterotrophic populations ranged from 5×10^2 cells/ℓ in July to 1.55×10^5 in April. Hydrocarbonoclastic bacterial populations were consistently very low, ranging from 0 to 12.9 cells/ℓ. Although the percentage of oil degrading bacteria ranged from 0 to 2.05%, it was usually less than 0.5%.

A total of 1,905 marine bacteria were isolated from the samples and 1,631 generic identifications have been completed at this time. Fourteen (14) genera were represented by the isolates. *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Flavobacterium*, *Pseudomonas*, and *Vibrio* were the only hydrocarbonoclastic genera isolated from any of the three stations. They were also prevalent in the heterotrophic bacterial isolates. *Alcaligenes*, *Aeromonas*, *Pseudomonas*, and *Vibrio* were the predominant genera for both the heterotrophs and hydrocarbonoclastic isolates. The greatest diversity of genera occurred at Station 3/II in April, with eight genera represented. Usually only three or four genera represented the bacterial populations in a given sample. *Pseudomonas* was the only genus represented in all 27 sample collections.

The highest percentage of oil degraders occurred in July and August when the heterotrophic population was decreased somewhat and the hydrocarbonoclastic populations were increased. Seasonal fluctuations in heterotrophs were most prevalent at Stations 1/II and 3/II, while the microbial populations at Station 2/II were most stable. In the winter season, the decreases in populations offshore was more pronounced. It was less apparent in the fall.

Some experiments conducted on the effects of South Louisiana crude oil (SLCO) on pure cultures and mixed bacterial cultures showed concentrations of 0.1% and 0.5% enhancing microbial growth even after four to six weeks. Temperature effects on microbial density was indicated, with the greatest populations occurring between temperatures of 23.8 to 26°C. The relationship of salinity to bacterial density in the water column seems inconclusive at this time.

The major genera in the succession studies represented, either in respect to number or frequency of occurrence, were *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, and Coryneforms. The presence or absence of SLCO in incu-

bating seawater samples was responsible for little, if any, change in total number of organisms or successful changes. Bacterial populations in both cases changed drastically within 7 to 10 days in the closed systems of the laboratory. Counts of ~ 10^5 to 10^6 colony forming units (CFU) per milliliter total heterotrophs and ~ 10^3 to 10^4 CFU/ml of hydrocarbonoclasts were found. However, it appeared that in time, deviation in genera of bacteria comprising these populations occurred. These changes did not seem to be due to the SLCO. It was also evident from hydrocarbon analysis and turbidimetric measurements that the metabolism of oil by bacteria in the Gulf of Mexico water was insignificant due to the low number of hydrocarbonoclasts and nutrient limitations.

The nutrient experiments showed that addition of nitrogen, phosphate, and iron, or a complete mineral salts medium significantly enhanced hydrocarbon metabolism. The total heterotrophic count increased 2^+ orders of magnitude over those samples without nutrients or oil. The hydrocarbon analysis performed on samples in time indicated an almost complete removal or metabolism of some n-alkanes.

The kinetic studies involved using a limited number of marine isolates and showed the optimum temperature for growth was between 31 and 41°C. The various combinations of temperature and oil stress produced no substantial changes when compared to the temperature controls without oil. The SLCO did not, in any case, enhance the growth time. However, the oil at the concentrations tested did not appear to produce significant inhibition.

The above conclusions are based on limited statistical treatment.

INTRODUCTION

The water column microbiology baseline study was initiated in 1977 as a part of the BLM South Texas Outer Continental Shelf (STOCS) program. The purpose of these investigations was to quantitate and identify the heterotrophic and hydrocarbonoclastic microbial populations, to study ecological succession of mixed natural bacterial populations in the presence of South Louisiana Crude Oil (SLCO), to study oil degradation potential of the mixed bacterial populations, and to study the effects of crude oil on bacterial growth and metabolism.

Although the levels of pollution resulting from large-scale oil spills have been high, such disasters account for only 4% of the total oil entering aquatic systems. Chronic inputs of low concentrations of crude oil and refined petroleum products account for the remaining 96%. These low level inputs are the results of normal operations involved in the production, transport use, and disposal of petroleum hydrocarbons. Natural effects which might be responsible for the clean-up could include biodegradation of oil by microorganisms in the environment. Studies involving the relationship of the presence of hydrocarbons in the environment and microbial communities adapted to the degradation of hydrocarbons have been conducted largely in areas subjected to continuous and appreciable petroleum hydrocarbon contamination. For this reason, it is important to know the indigenous potential for the petroleum degradation.

Naturally occurring marine microorganisms have been shown to oxidize and degrade Louisiana crude oil (Miget *et al.*, 1969; Kator *et al.*, 1971). The microbes preferentially degraded the normal saturated paraffins. Prichard *et al.* (1976) investigated the use of diesel fuel as a substrate for mixed bacterial populations. They found that both alkanes and aromatics were removed from the culture by microbial degradation. Certain species of bacteria are highly specialized for growth on crude oil and they will only utilize certain hydrocarbons, but other species can utilize numerous different carbon compounds (Horowitz *et al.*, 1975). Mulkins-Phillips and Stewart (1974) showed mixed bacterial populations could degrade Bunker C fuel oil. Other similar studies have been conducted in the Chesapeake Bay Region (Walker and Colwell, 1976), the Arctic (Atlas and Busdosh, 1976; Roubal and Atlas, 1978), the Eastern Sea Coast (Kator, Oppenheimer and Miget, 1971), and the Western Sea Coast (Soli and Bens, 1972). However, few studies have been conducted on the Western Gulf of Mexico to identify the normal microbial populations and to determine their oil degradation potential. Environmental factors such as temperature, salinity, nitrogen, phosphorus, iron, and other mineral nutrients, in addition to oxygen, are very important in establishing the rate of bacterial oil degradation (Mulkins-Phillips and Stewart, 1974; Soli and Bens, 1972; Atlas and Busdosh, 1976; Dibble and Bartha, 1976). If the proper conditions are available, the rate of utilization can be very rapid.

Due to the fact that few studies of this type have been conducted on the Western Gulf of Mexico, this study was initiated to provide microbiological data for future comparisons. This report presents the data obtained in this initial year's study with some correlations with a few environmental factors.

MATERIALS AND METHODS

Shipboard Procedures

Sampling

Water samples for the water column microbiological studies were collected aseptically using one of two procedures. For smaller volumes, the sterile Niskin bag sampler was used; however, for larger volumes of water (15-20 ℓ) a peristaltic pump and tygon tubing were employed. The collections were made at a depth of one meter at Stations 1/II, 2/II and 3/II during six monthly and three seasonal cruises, for a total of 27 samples (Table 8.1).

Analysis

After transferring the samples aseptically to sterile carboys, the initial plating and inoculations of the marine microorganisms on microbiological media were made. In order to determine the total viable cells (TVC), or colony forming units (CFU), of aerobic heterotrophic bacteria, both the spread plate technique and the membrane filter technique were employed. Ten-fold dilutions of the water samples were prepared to 1:10 dilution, and 0.1 ml aliquots of each of the two dilutions were plated out, in triplicate, on Marine Agar 2216 (MSA) and Difco Marine Agar 2216 containing 10 μg/ml Fungizone (FAM), using the spread plate technique. Fungizone was incorporated into the medium in order to eliminate yeasts and molds from the cultures. Microbial populations of the water samples were also concentrated by membrane filtration in order to assure collection of adequate numbers of heterotrophic bacteria. Bacteria from 0.1, 1.0, 10 and 50 ml of seawater sample were collected (in triplicate) on sterile membrane filters, which were then aseptically transferred to MSA and FAM plates.

In order to assure collection of an adequate number of potential hydrocarbonoclastic (petroleum degrading) bacteria, aliquots of 100, 500, 1000, and 2000 ml of water sample were passed through sterile membrane filters. The filters were then transferred aseptically to Silica Gel Oil (SGO) medium and Silica Gel Oil medium with 10 μg/ml Fungizone (FOG) plates (Walker and Colwell, 1976). Triplicate samples were prepared for each volume of water used.

TABLE 8.1

CRUISE DATES

Cruise	Date
Winter Seasonal	2/17/77
March Monthly	3/25/77
April Monthly	4/25/77
Spring Seasonal	6/10/77
July Monthly	7/9/77
August Monthly	8/6/77
Fall Seasonal	10/21/77
November Monthly	11/20/77
December Monthly	12/16/77

All plates were returned to the laboratory and incubated at *in situ* temperatures. The MSA and FAM plates (for heterotrophic bacteria) were incubated a minimum of seven days, and the SGO and FOG plates (for hydrocarbonoclastic bacteria) were incubated a minimum of seven weeks. These incubation times were found to be necessary to assure growth of the maximum colonies (Figures 8.1 and 8.2). Enumeration results were recorded as total number of heterotrophic microorganisms per liter, total hydrocarbonoclastic microorganisms per liter, and percent oil degraders.

Studies of the hydrocarbon degradation potential and microbial succession of mixed cultures were initiated on board ship. For each station (at each location, during each cruise) at least 12 flasks (250 ml capacity) were used. Table 8.2 lists the scheme required for the succession studies and the oil degradation studies. Four additional flasks were collected at Station 1/II. Four additional flasks per station were used during the first three cruises to get a better estimate of the time course of the degradation.

Each flask with oil contained a total volume of 100 ml, usually 99.5 ml seawater plus 0.5 ml sterile SLCO. The oil concentration was reduced to 0.1% on August, Fall, November and December cruises. Those flasks without oil had 100 ml seawater. The flask treatments were as follows, and are given in Table 8.2.

Except for the first three cruises, six flasks containing SLCO were used for studying the bacterial succession with oil added. Six flasks did not have any oil and were used as the bacterial controls. Six flasks had SLCO and were used for chemical analysis. Six flasks were extras; three had oil and three were potential bacterial controls. Two sterile weathering controls and two zero time controls were also collected at Station 1/II for chemical analysis.

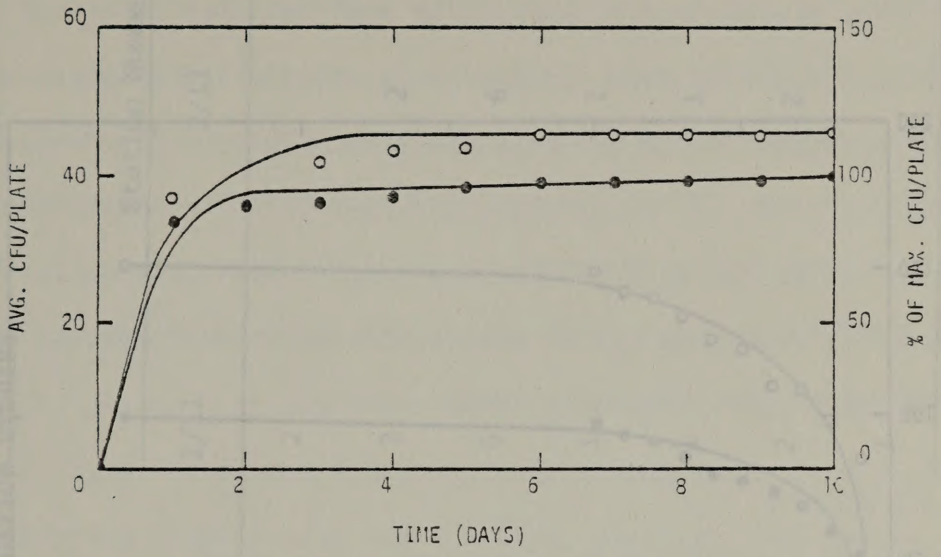


Figure 8.1 Time Required to Obtain Maximum Colony Counts on Marine 2216 Media. Bacteria were Plated from Seawater Samples Incubated Without Oil in the Laboratory on a Rotary Shaker at 25°C. Open Circles Represent the Actual CFU (Colony Forming Units), and the Closed Circles Represent the Percent of the Maximum CFU.

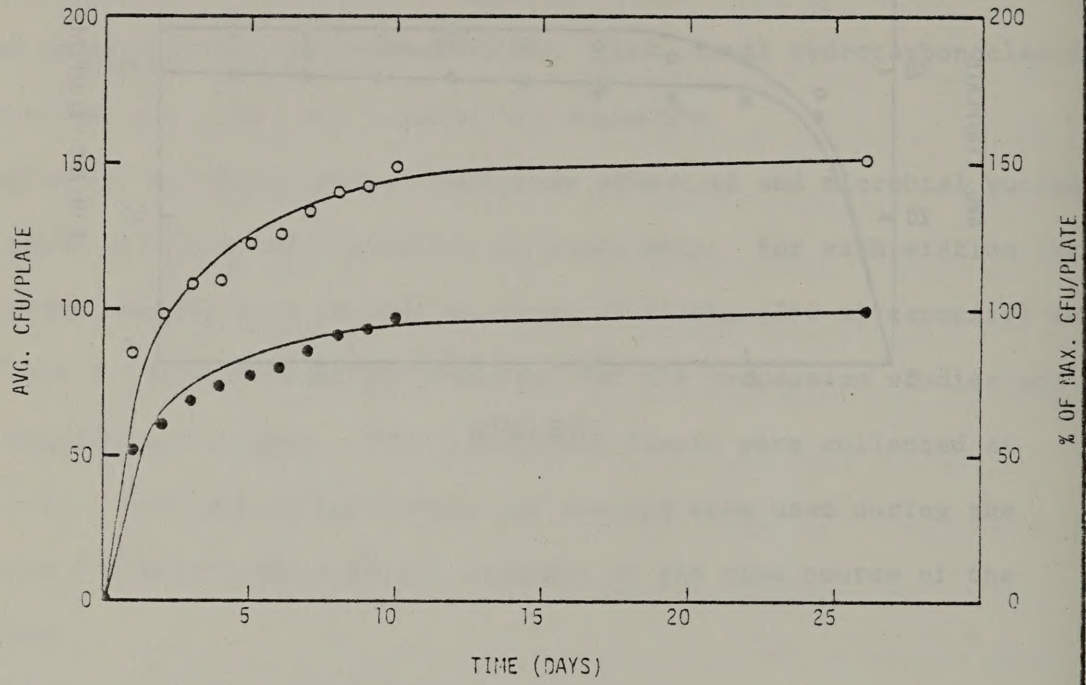


Figure 8.2 Time Required to Obtain Maximum Colony Counts on Marine 2216 Media. Bacteria were Plated from Seawater Samples Incubated with 0.5% South Louisiana Crude Oil on a Rotary Shaker at 25°C. Open Circles Represent the Actual CFU (Colony Forming Units), and the Closed Circles Represent the Percent of the Maximum CFU.

TABLE 8.2

NUMBER OF FLASKS PREPARED ON BOARD SHIP FOR THE BACTERIAL SUCCESSION STUDIES AS WELL AS THE OIL DEGRADATION STUDIES

Treatment	Station Number		
	1/11	2/11	3/11
Time Zero (SLCO)* (frozen)	2		
Bacterial Analysis (SLCO)	2	2	2
Chemical Analysis (SLCO)	6	6	6
Extra A. (SLCO)	1	1	1
B. Control	1	1	1
Bacterial Control (No oil)	2	2	2
Weathering Control (sterile-SLCO)	2		
Totals	16	12	12

*Samples marked with SLCO contained o.1-0.5% South Louisiana crude oil.

After the water sample was added, the flasks were carefully capped. Aseptic techniques were used at all times. The inoculated flasks were returned to the laboratory and incubated at room temperature (20-50°C) on a reciprocal shaker for six to eight weeks or more to determine the microbial succession, as well as the hydrocarbon degradation capacity of the mixed culture. All flasks were sterilized by autoclaving prior to use in these studies. Added oil was filter sterilized with a 0.45 μ Millipore Filter$^{(R)}$ prior to use. Zero controls were placed in ice immediately after collection, and then frozen on return to the laboratory. Mercuric chloride (0.1 g) was added to the eight week weathering controls to prevent microbial activity during the incubation period.

Laboratory Procedures

Isolation and Identification of Heterotrophic and Hydrocarbonoclastic Bacteria

Plates used for the enumeration studies were used as the source of heterotrophic and hydrocarbonoclastic bacteria, for isolation and identification of the bacterial genera. For the identification procedures, a minimum of 30 colonies were picked from one or more of the MSA or FAM plates depicting the best isolated colonies for each of the three stations sampled. For isolation of the heterotrophic bacteria, all the colonies were picked from a single plate, or a quadrant of the more heavily populated plates. All oil degrading bacteria enumerated on the SGO and FOG plates were isolated and identified. The heterotrophs were transferred directly to Marine Broth 2216, incubated at room temperature (with shaking) for a minimum of 24-48 h prior to being restreaked on Marine Agar 2216 to assure purity of the isolates. After purity was established, the heterotrophs were maintained on MSA slants for future studies.

For the hydrocarbonoclastic isolates, the organisms were transferred from the original FOG or SGO plates to SGO plates to assure purity and hydrocarbon utilization by the isolate. Once this was established, the petroleum degraders were also inoculated in Marine Broth and then transferred to Marine Agar slants for maintenance for further tests.

The bacterial isolates were identified to the generic level according to their microscopic, morphological, and biochemical characteristics, as outlined in the Eighth Edition of Bergey's Manual of Determinative Bacteriology. Approximately 15-20 tests were conducted for each isolate, depending upon the specific genus being tested. Some of the differential media and tests employed in the identification of the bacterial isolates were litmus milk, gelatin, triple sugar iron, tryptone broth, Simmons citrate agar, urea broth, ornithine decarboxylase, carbohydrate fermentation media, marine oxidative fermentative (MOF) medium, motility test medium, catalase test, and oxidase test. All isolates were Gram stained and flagella stained.

Most of the marine isolates were identified according to Bergey's Manual. However, other references specifically concerned with bacteria of marine origin were also employed in the bacterial identifications (Baumann *et al.*, 1971a; Baumann *et al.*, 1971b; Colwell, 1970; Floodgate and Hayes, 1963; Hayes, 1963; Stanier *et al.*, 1966).

Effects of Oil on Pure and Mixed Cultures

For the studies involving pure cultures, 100 ml artificial seawater with nitrogen and phosphorus supplements, was added to 250 ml screw cap flasks, and inoculated with the appropriate amount of SLCO to give final concentrations of 0.01%, 0.05%, 0.1%, 0.5%, 1.0%, and 2.0% as required for the specific experiment. After the flasks were sterilized and cooled they were inoculated with the appropriate isolate.

For the mixed culture-oil studies, seawater samples were used in place of the artificial seawater, and also provided the bacterial inoculum for the study. The flasks were incubated at room temperature (with shaking) for eight weeks. All flasks were sampled at regular intervals to determine the TVC/ml.

Succession Studies

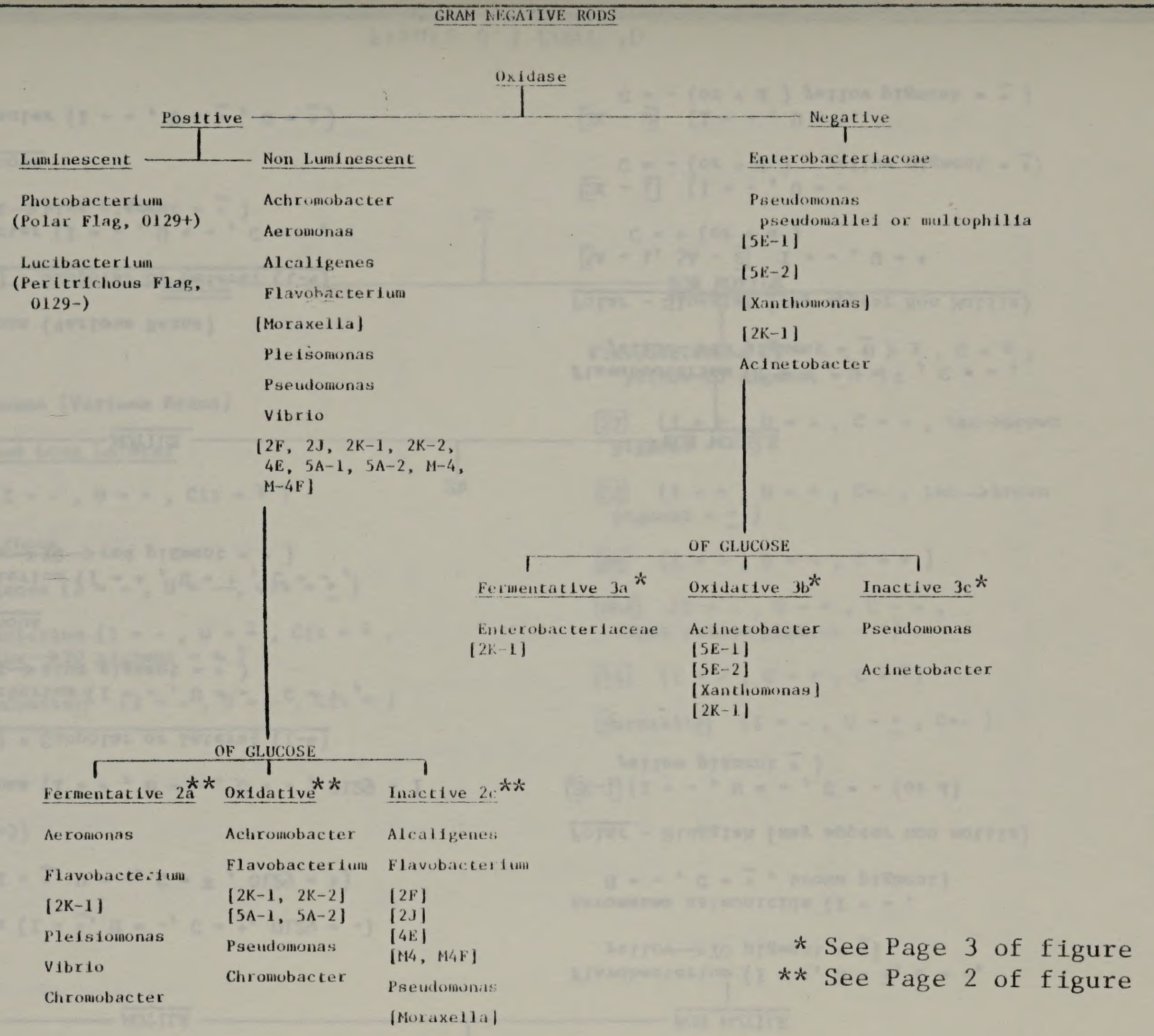
Isolation of Organism

Morphologically distinct colonies of the heterotrophs and hydrocarbon utilizing bacteria found on the 2216 or SGO plates were counted, picked, and streaked on 2216 media for isolation. After the purity of the isolates was assured by restreaking, the isolates were maintained on slants of the 2216 medium.

Identification of Organisms

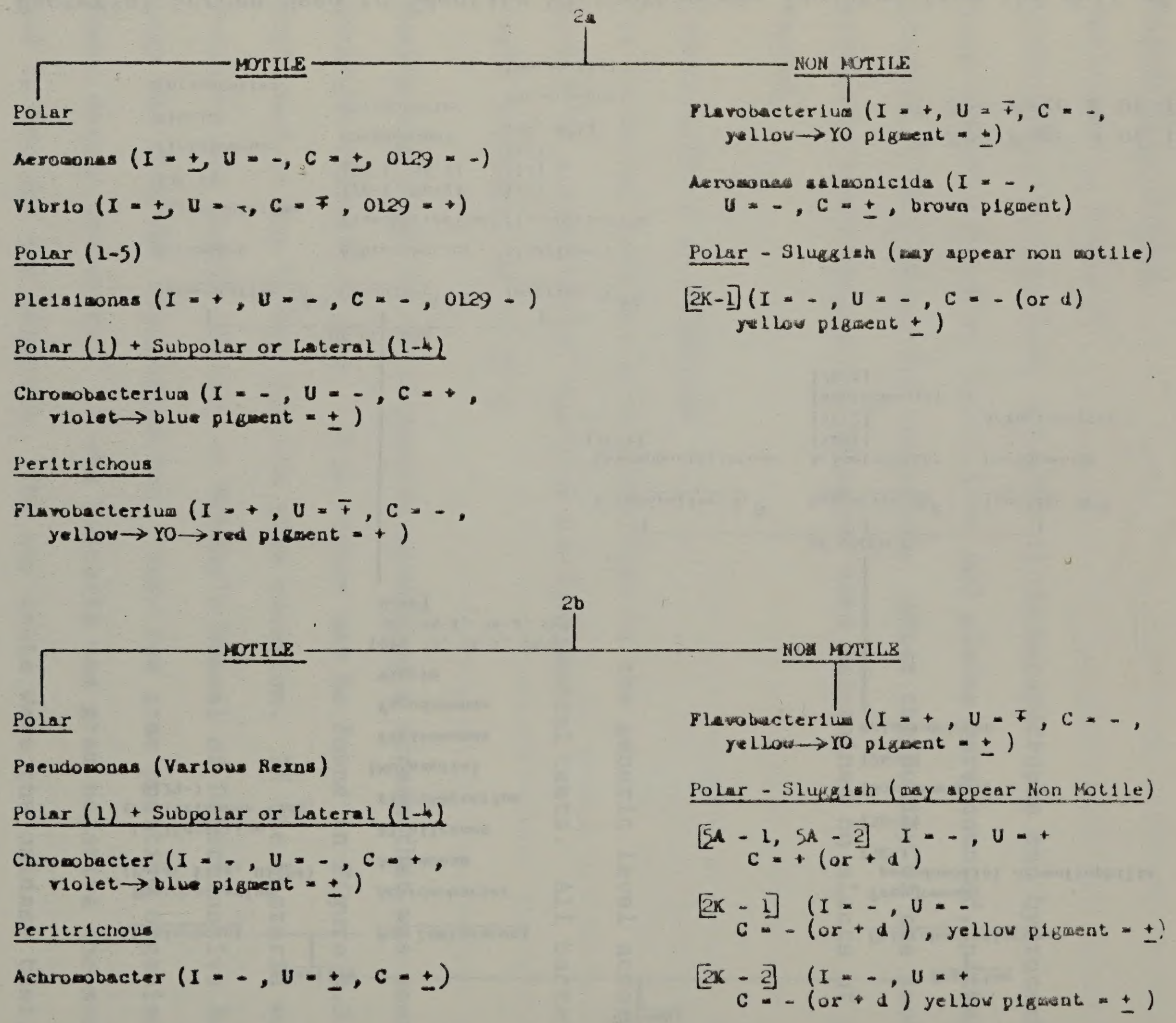
The bacterial isolates were identified to the generic level according to a number of morphological features and biochemical tests. All bacterial tests were incubated at 20-25°C.

During the study Dr. Neal Guntzel developed a screen that was used for the bacterial identifications. This screen may be found in Figure 8.3. The first test in the screen was the gram reaction. If the bacteria was gram positive the eighth edition of Bergey's Manual of Determinative Bacteriology was used for identification. Very few gram positive organisms were found during the study. If the bacteria was gram negative the screen was used to key out the organism. The key tests were the oxidase test, oxidative-fermentative (OF) glucose, motility and luminescence. Other tests used to key out the genera are found in Table 8.3. The bacterial screen that was developed and used was quite simple, it used key fixed characteristics, which are not easily changed, and it was easy to use and saved considerable time. A considerable effort was expended developing



* See Page 3 of figure
** See Page 2 of figure

Figure 8.3 Bacterial Screen Used to Identify Microorganisms Isolated from the Gulf of Mexico.



2a

MOTILE

Polar

Aeromonas (I = ±, U = -, C = ±, 0129 = -)

Vibrio (I = ±, U = -, C = ∓ , 0129 = +)

Polar (1-5)

Pleisimonas (I = + , U = - , C = - , 0129 -)

Polar (1) + Subpolar or Lateral (1-4)

Chromobacterium (I = - , U = - , C = + , violet → blue pigment = ±)

Peritrichous

Flavobacterium (I = + , U = ∓ , C = - , yellow → YO → red pigment = +)

NON MOTILE

Flavobacterium (I = +, U = ∓, C = -, yellow → YO pigment = ±)

Aeromonas salmonicida (I = - , U = - , C = ± , brown pigment)

Polar - Sluggish (may appear non motile)

[2K-1] (I = - , U = - , C = - (or d) yellow pigment ±)

2b

MOTILE

Polar

Pseudomonas (Various Rexns)

Polar (1) + Subpolar or Lateral (1-4)

Chromobacter (I = - , U = - , C = + , violet → blue pigment = ±)

Peritrichous

Achromobacter (I = - , U = ± , C = ±)

NON MOTILE

Flavobacterium (I = + , U = ∓ , C = - , yellow → YO pigment = ±)

Polar - Sluggish (may appear Non Motile)

[5A - 1, 5A - 2] I = - , U = + C = + (or + d)

[2K - 1] (I = - , U = - C = - (or + d) , yellow pigment = ±)

[2K - 2] (I = - , U = + C = - (or + d) yellow pigment = ±)

Figure 8.3 CONT.'D

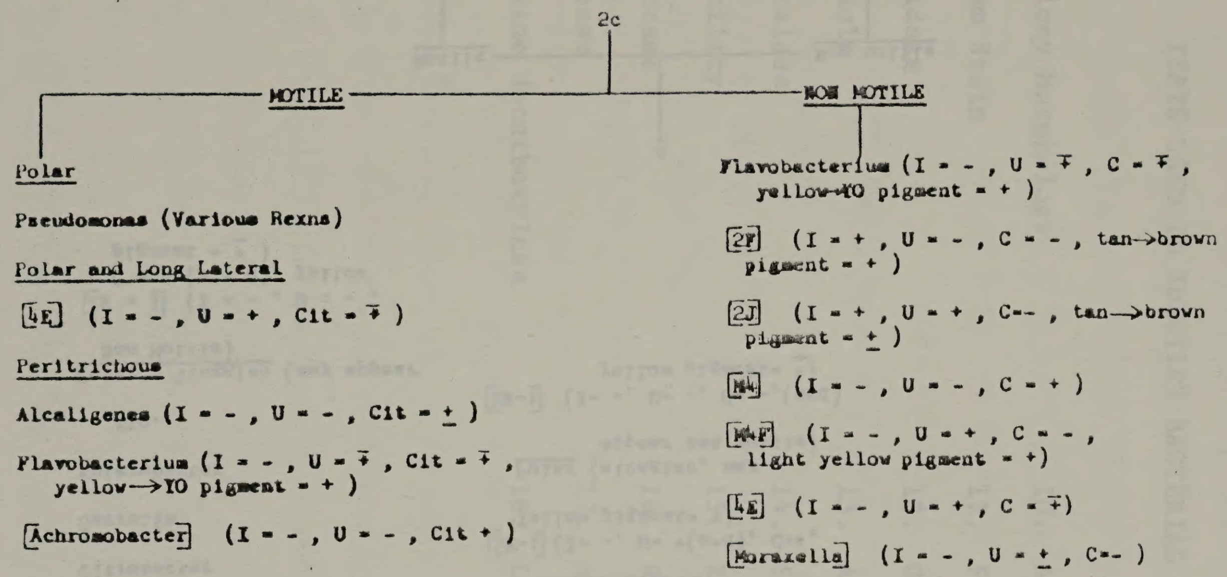

2c
MOTILE
NON MOTILE
Polar
Pseudomonas (Various Rexns)
Polar and Long Lateral
[4E] (I = - , U = + , Cit = ∓)
Peritrichous
Alcaligenes (I = - , U = - , Cit = ±)
Flavobacterium (I = - , U = ∓ , Cit = ∓ , yellow→YO pigment = +)
[Achromobacter] (I = - , U = - , Cit +)
Flavobacterium (I = - , U = ∓ , C = ∓ , yellow→YO pigment = +)
[2F] (I = + , U = - , C = - , tan→brown pigment = +)
[2J] (I = + , U = + , C=- , tan→brown pigment = ±)
[M4] (I = - , U = - , C = +)
[M4F] (I = - , U = + , C = - , light yellow pigment = +)
[4E] (I = - , U = + , C = ∓)
[Moraxella] (I = - , U = ± , C=-)

Figure 8.3 CONT.'D

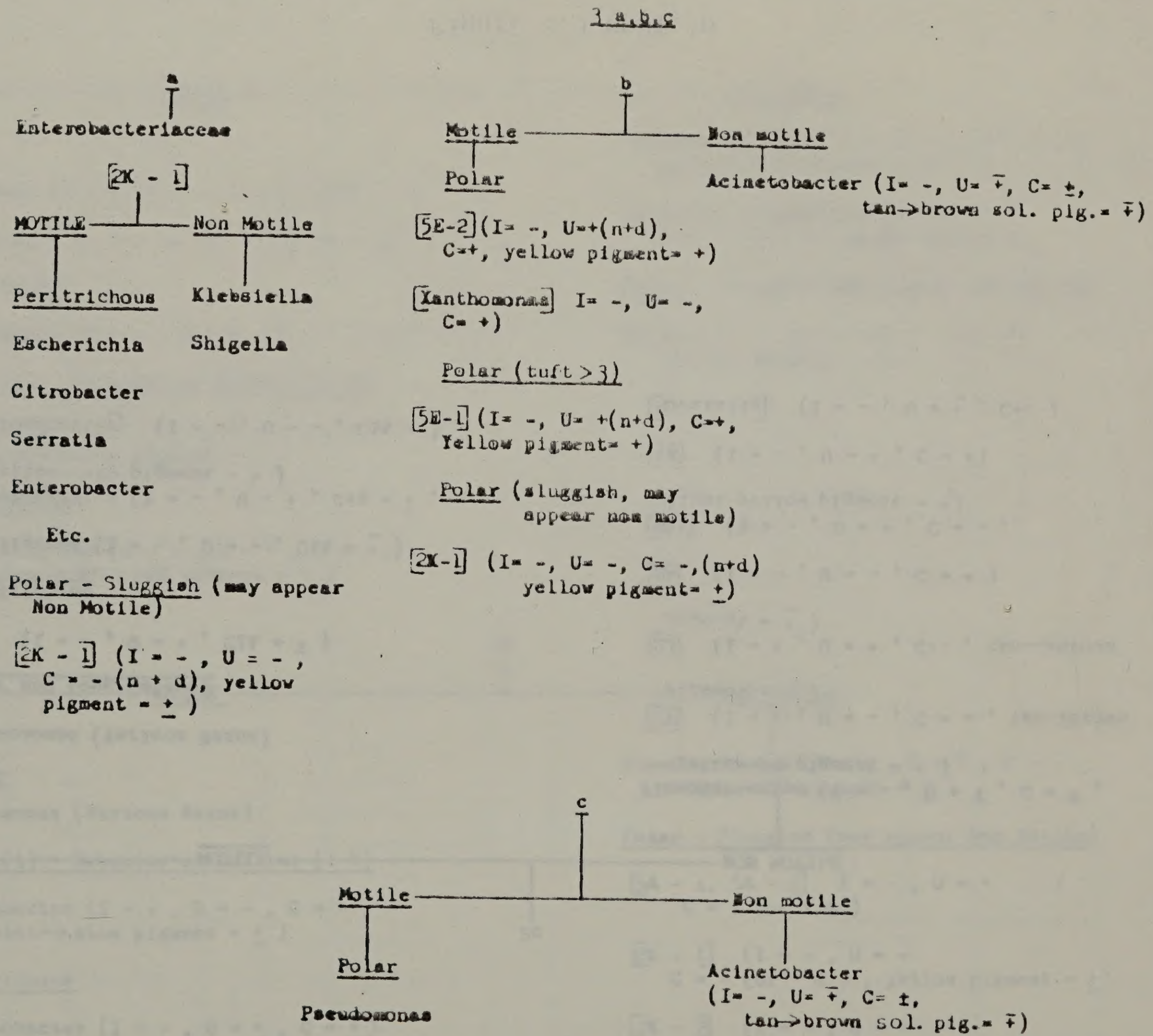

3 a,b,c
a
Enterobacteriaceae
[2K - 1]
MOTILE
Non Motile
Peritrichous
Klebsiella
Escherichia
Shigella
Citrobacter
Serratia
Enterobacter
Etc.
Polar - Sluggish (may appear Non Motile)
[2K - 1] (I = - , U = - , C = - (n + d), yellow pigment = ±)
b
Motile
Non motile
Polar
Acinetobacter (I= -, U= ∓, C= ±, tan→brown sol. pig.= ∓)
[5E-2] (I= -, U=+(n+d), C=+, yellow pigment= +)
[Xanthomonas] I= -, U= -, C= +)
Polar (tuft > 3)
[5E-1] (I= -, U= +(n+d), C=+, Yellow pigment= +)
Polar (sluggish, may appear non motile)
[2K-1] (I= -, U= -, C= -,(n+d) yellow pigment= ±)
c
Motile
Non motile
Polar
Pseudomonas
Acinetobacter (I= -, U= ∓, C= ±, tan→brown sol. pig.= ∓)

Figure 8.3 CONT.'D

TABLE 8.3

TESTS USED TO IDENTIFY BACTERIAL ISOLATES

1. Colony Morphology
2. Gram Stain
3. Oxidase
4. Indole
5. Catalase
6. Motility
7. Citrase
8. Urease
9. Lysine decarboxylase
10. Arginine dihydrolase
11. Fluorescence
12. Glucose
13. Arabinose
14. Saccharose
15. Xylose
16. Rhamnase
17. Adonitol
18. Lactose

the screen.

Two additional method simplifications were integrated into the identification scheme. To reduce costs and space requirements and to speed up handling of the numerous bacterial strains, multipoint applicators similar to those described by Lovelace and Colwell (1968) and microtest plates were used. This type of automation and miniaturization allowed a 30-60% reduction in the time required to read most of the biochemical tests used for bacterial identification. Also, 96 tests were run on a single plate (Figure 8.3) reducing the amount of media required as well as the number of plates required for microbial identification.

Degradation Rates

To determine the degrading potential of pure cultures of the isolated microorganisms, procedures similar to those previously used for determining the degradation potential of the mixed cultures in seawater were employed. Duplicate flasks containing 100 ml of marine basal salts medium and 0.01 - 1.0% (v/v) SLCO were inoculated with the individual isolates. All flasks were incubated six to eight weeks on a reciprocal shaker at temperatures ranging from 15 - 45°C. At appropriate intervals, bacterial growth was evaluated by turbidity measurements, and by plating samples on marine agar. Gas chromatography analysis was also conducted on selected samples at appropriate intervals to establish the rate of hydrocarbon degradation of the isolates.

Mixed culture degradation studies initiated on board ship were also evaluated at appropriate intervals with regard to total CFU, microbial succession, and rate and pattern of hydrocarbon degradation.

Nutrient Studies

For a selected number of mixed cultures, several parameters were adjusted under controlled laboratory conditions to represent variations which might occur at the sampling site. The effect of nitrogen, phosphate, iron, and organic concentrations on growth rates and generation times were determined. Other turbidimetric studies using a Spectronic 20 set at 600 nm and 25 x 250 mm capped growth tubes were used to determine ratios of known media on oil degrading capacities. Reaction mixtures usually contained 10 ml of raw seawater, an aliquot of Bushnell-Hass (BH) medium, an aliquot of 3.5% NaCl to make the total sample volume equal 20 ml plus 0.0 - 0.5% SLCO.

Extraction and Chromatographic Analyses

Oil-water suspensions used for bacterial hydrocarbon degradation analyses and determining the rates of degradation were frozen and shipped to Dr. P.L. Parker at the UTMSI/PAML for extraction and chromatographic analysis. Printouts were returned after quantitation for inspection and statistical treatment.

Kinetic Studies

Effects of Oils on Microbial Populations

SLCO was used to investigate the effects of hydrocarbon concentrates on certain mixed populations and certain isolates. Growth rates, oxidation rates, and succession, where appropriate, were determined.

The SGO media as described by Walker and Colwell (1976) was modified and had the components listed in Table 8.4. We found during the study that at pH 8.1, it was necessary to add 0.5 g/ℓ of Fe citrate for $FeCl_3$ in order to adequately enumerate the hydrocarbon degraders (Figure 8.4). When iron was used the maximum number of colonies could be counted after 15 days (Figures 8.5 and 8.6). Other experiments suggested that lowering the pH of the media (toward the neutral range 7.0 - 7.5) would allow much smaller

TABLE 8.4

COMPONENTS OF THE SILICA GEL-OIL MEDIA REQUIRED TO MAKE TWO LITERS OF MEDIA

Solution A		Solution B	
1000 ml Salts Solu.		1000 ml Silica Gel Solu.	
Amount	Component	Amount	Component
2 gm	KCl	60 gm	Fisher Grade 923 Silica gel (100-200 mesh)
2 gm	NH_4NO_3		
8 gm	$MgSO_4$	40 gm	NaOH
2 gm	kH_2PO_4	1000 ml	H_20
.03 gm	Phenol Red Indicator		
10 ml	South Louisiana Crude OIL		
0.5 gm	$FeCl_3$		
1000 ml	H_20		

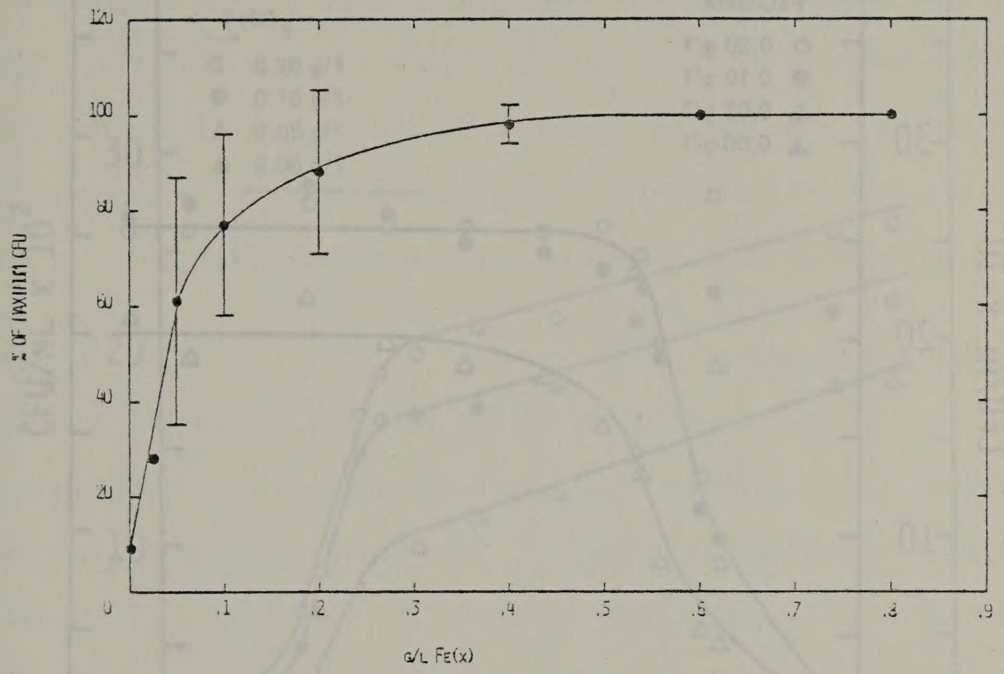


Figure 8.4 Dependence of Determination of Maximum CFU on the Fe(x) Concentration Present in the SGO Media. Points Represent the Average of Three Replicate Experiments ± 1 SD.

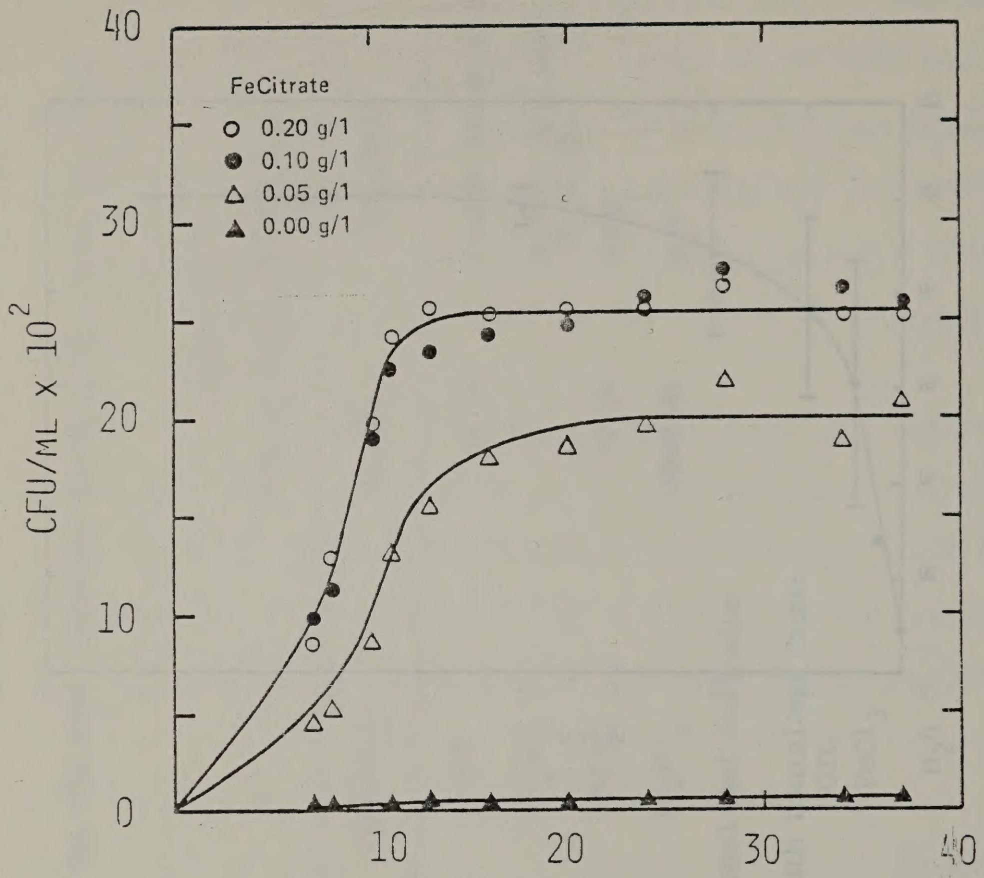


Figure 8.5 The Effect of Additions of Ferric Citrate on the Number of Bacterial Colonies (CFU/ml) Found on SGO Media.
▲ = 0.00 g/ℓ, △ = 0.05 g/ℓ, ● = 0.10 g/ℓ, ○ = 0.20 g/ℓ

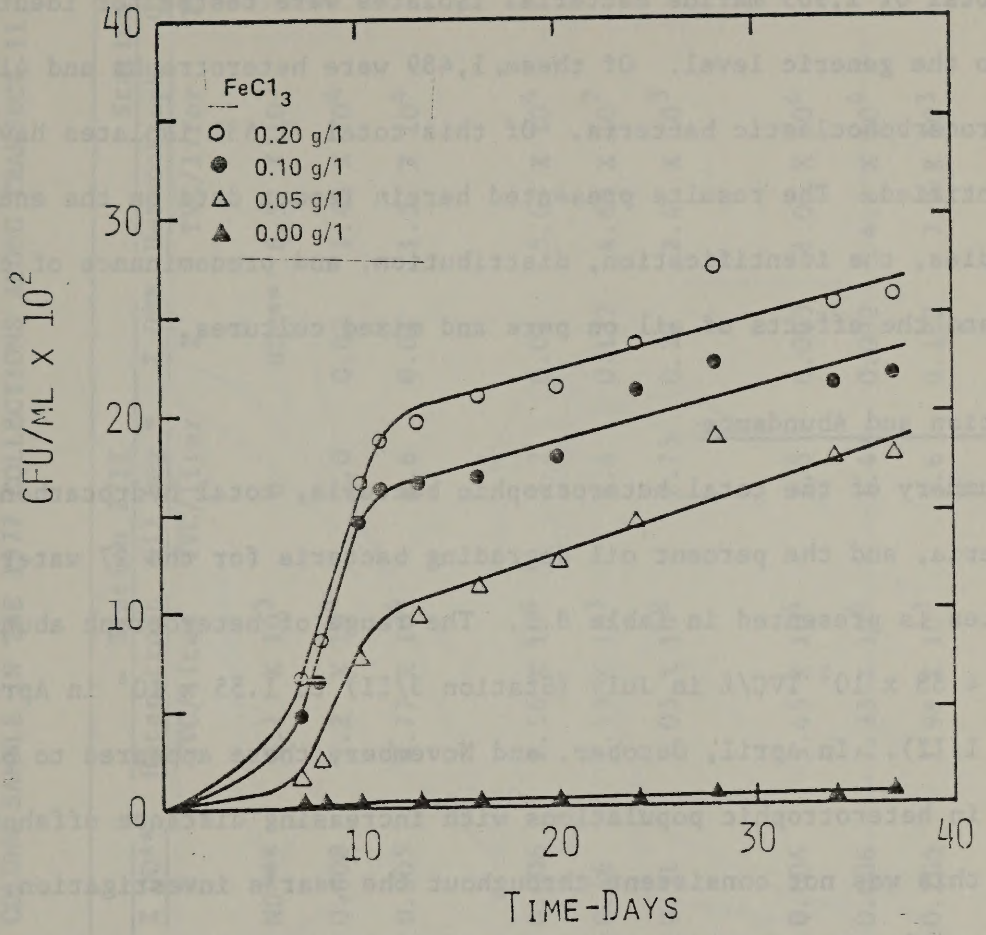


Figure 8.6 The Effect of Additions of Ferric Chloride on the Number of Bacterial Colonies (CFU/ml) Found on SGO Media.
▲ = 0.00 g/ℓ, △ = 0.05 g/ℓ, ● = 0.10 g/ℓ, ○ = 0.20 g/ℓ

amounts of iron to be added to the media and still obtain rapid growth of the microbial colonies.

RESULTS

A total of 1,905 marine bacterial isolates were tested for identification to the generic level. Of these, 1,489 were heterotrophs and 416 were hydrocarbonoclastic bacteria. Of this total, 1,631 isolates have now been identified. The results presented herein report data on the enumeration studies, the identification, distribution, and predominance of certain genera, and the effects of oil on pure and mixed cultures.

Distribution and Abundance

A summary of the total heterotrophic bacteria, total hydrocarbonoclastic bacteria, and the percent oil degrading bacteria for the 27 water column samples is presented in Table 8.5. The range of heterotroph abundance was from 4.88×10^2 TVC/ℓ in July (Station 3/II) to 1.55×10^5 in April (Station 1/II). In April, October, and November, there appeared to be a decrease in heterotrophic populations with increasing distance offshore. However, this was not consistent throughout the year's investigation. Hydrocarbonoclastic populations were consistently very low, ranging from 0 to 12.9 TVC/ℓ. Except for the 2.05% oil degraders recorded for Station 3/II, in July, the other percentages of oil degraders were significantly less than 0.5%. Figures 8.7, 8.8 and 8.9 show the monthly variations of heterotrophic bacterial abundance, hydrocarbonoclastic bacterial abundance, and percentage of hydrocarbonoclastic bacteria. A comparison of these three figures indicates that the significant increase in percentage of oil degraders in July and August resulted from two phenomena. First, heterotrophic populations at all three stations decreased during July. Secondly, there was a sharp increase in the hydrocarbonoclastic populations at Sta-

TABLE 8.5

TOTAL HETEROTROPHIC BACTERIA, TOTAL HYDROCARBONOCLASTIC BACTERIA, AND PERCENT OIL DEGRADERS FOR EACH WATER COLUMN SAMPLE IN THE 1977 COLLECTIONS ALONG TRANSECT II.

Collection Time	Station 1/II			Station 2/II			Station 3/II		
	Heterotroph	Oil Deg.*	% OD**	Heterotroph	Oil Deg.*	% O**	Heterotroph	Oil Deg.*	% OD**
	TVC/liter	TVC/Liter	%	TVC/liter	TVC/liter	%	TVC/liter	TVC/Liter	%
February	1.1 X 10^5	0	ND***	6.1 X 10^3	0	ND***	6.5 X 10^3	10.0	0.154
March	1.5 X 10^5	12.9	0.008	1.2 X 10^4	2.0	0.02	1.48 X 10^4	0.67	0.005
April	1.55 X 10^5	8.5	0.005	4.77 X 10^4	4.6	0.01	1.5 X 10^4	1.5	0.01
June	4.3 X 10^4	2.4	0.006	2.56 X 10^4	2.2	0.01	5.67 X 10^4	4.0	0.007
July	5.26 X 10^2	2.4	0.46	7.15 X 10^3	9.4	0.132	4.88 X 10^2	10.0	2.05
August	2.03 X 10^3	6.2	0.31	1.03 X 10^3	2.75	0.27	2.4 X 10^3	1.5	0.063
October	9.2 X 10^4	3.6	0.004	5.45 X 10^4	1.5	0.003	1.06 X 10^4	1.13	0.011
November	8.97 X 10^4	5.0	0.006	7.33 X 10^4	1.42	0.002	4.8 X 10^4	1.84	0.004
December	8.53 X 10^2	1.75	0.205	1.98 X 10^3	3.6	0.182	7.9 X 10^3	0.75	0.0095

* Oil Degrading, or hydrocarbonoclastic bacteria.

** Percent Oil Degraders $= \dfrac{\text{Number of Hydrocarbonoclastic Bacteria}}{\text{Number of Heterotrophic Bacteria}} \times 100$

*** Not determined.

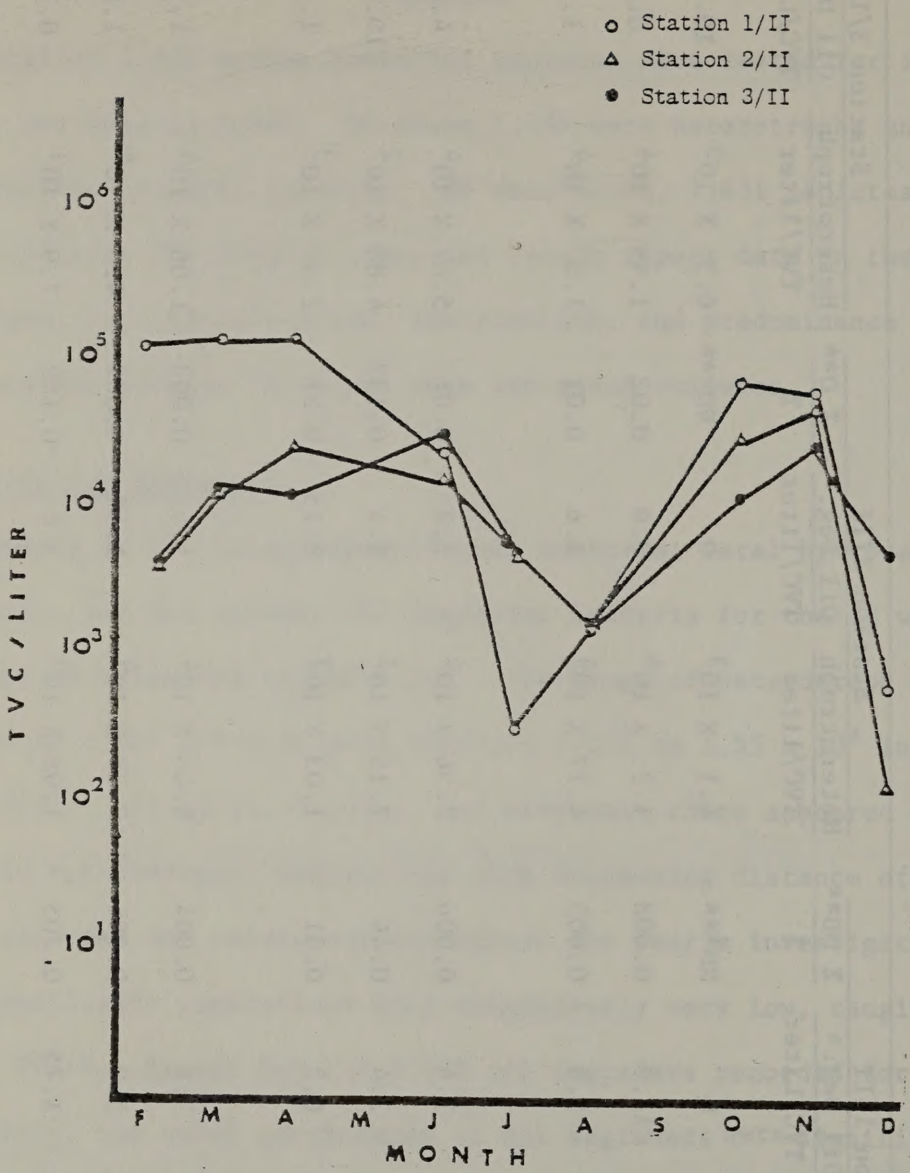


Figure 8.7 Monthly Variations of Heterotrophic Bacterial Abundance (cells l^{-1}) Along Transect II in the Water Column at a Depth of One Meter.

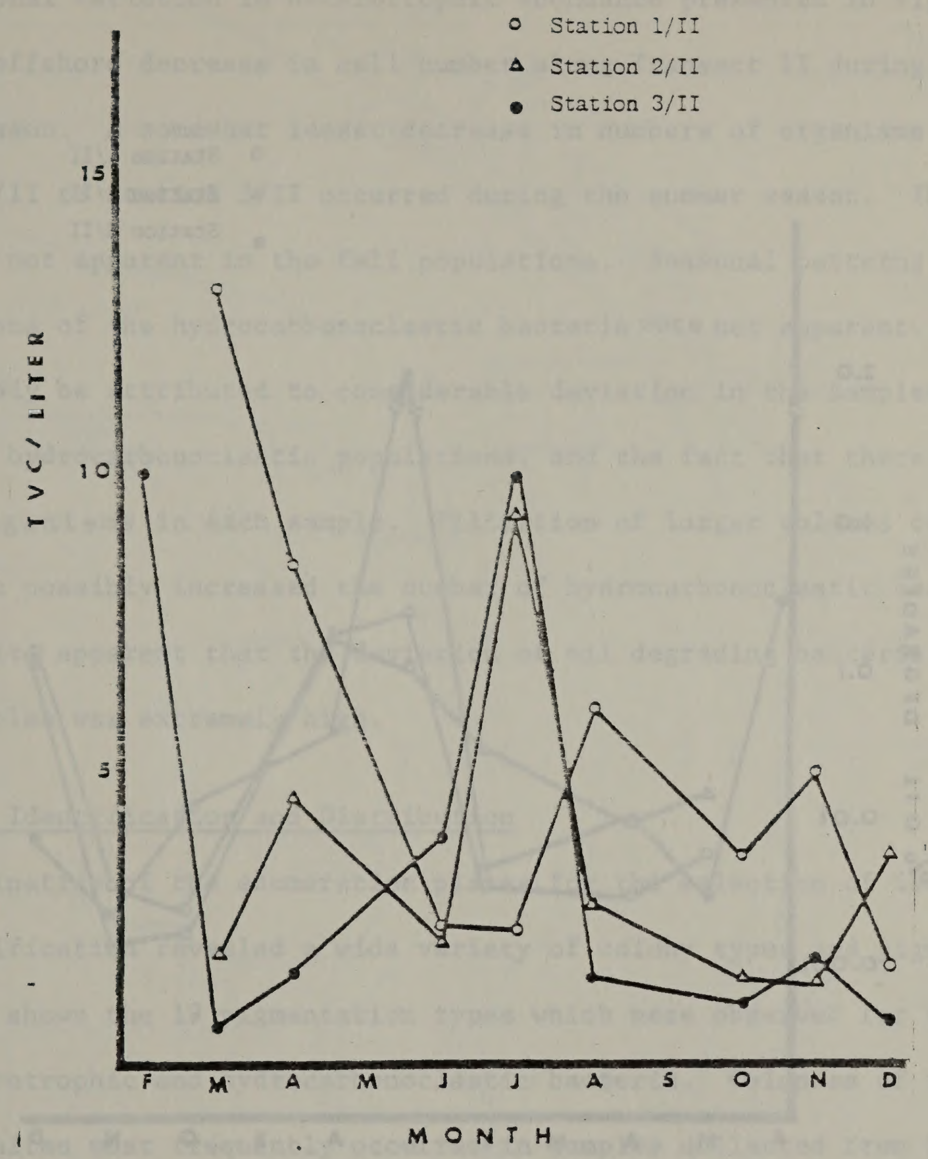


Figure 8.8 Monthly Variation of Hydrocarbonoclastic Bacterial Abundance Along Transect II at One Meter's Depth in the Water Column.

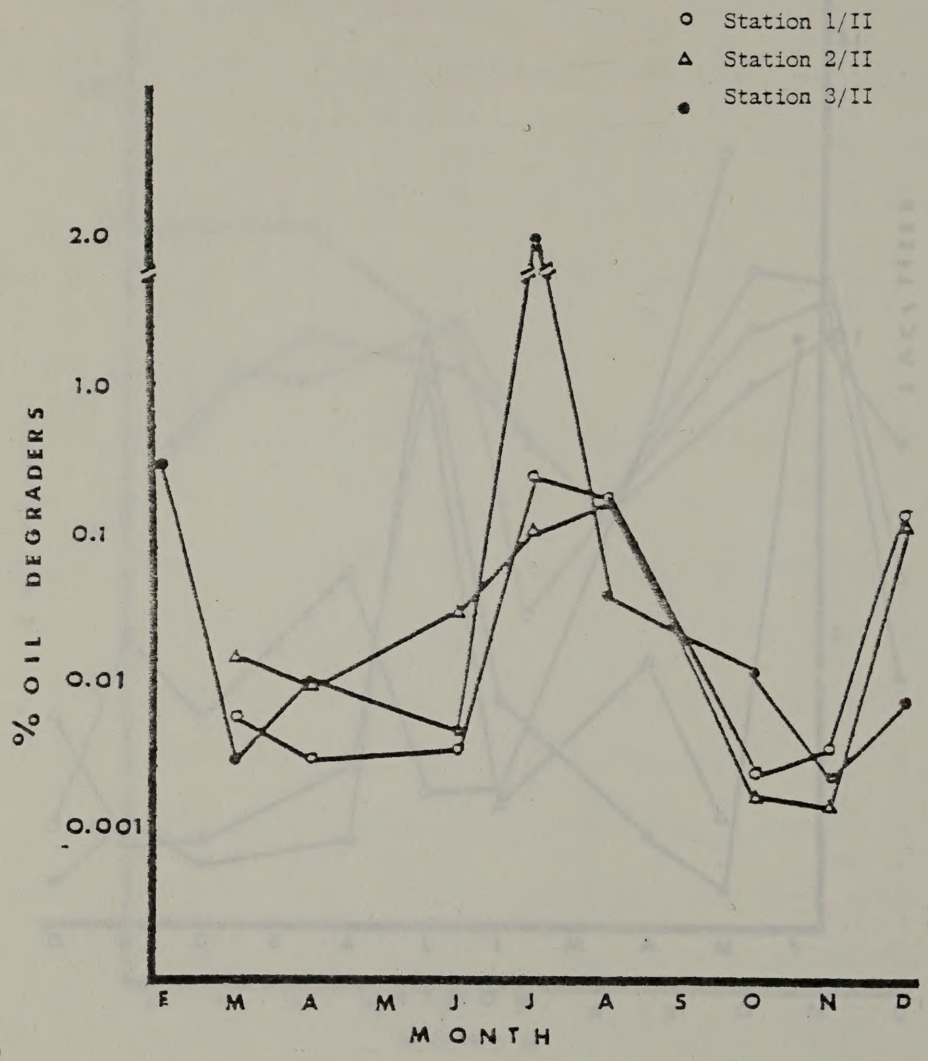


Figure 8.9 Monthly Variation of the Percentage Hydrocarbonoclastic Bacteria Along Transect II at One Meter's Depth in the Water Column.

tions 2/II and 3/II. The monthly variations in heterotrophic populations at Stations 2/II and 3/II were quite similar throughout the year.

Seasonal variation in heterotrophic abundance presented in Figure 8.10 shows an offshore decrease in cell number along Transect II during the winter season. A somewhat lesser decrease in numbers of organisms from Station 1/II to Station 3/II occurred during the summer season. This trend was not apparent in the fall populations. Seasonal patterns in the fluctuations of the hydrocarbonoclastic bacteria were not apparent. This may possibly be attributed to considerable deviation in the samples with regard to hydrocarbonoclastic populations, and the fact that there were so few organisms in each sample. Filtration of larger volumes of water could have possibly increased the number of hydrocarbonoclastic bacteria. It was quite apparent that the deviation of oil degrading bacteria in the water samples was extremely high.

Bacterial Identification and Distribution

Examination of the enumeration plates for the selection of isolates for identification revealed a wide variety of colony types and pigmentation. Table 8.6 shows the 19 pigmentation types which were observed for the various heterotrophic and hydrocarbonoclastic bacteria. Colonies of the whites and off-whites most frequently occurred in samples collected from Station 1/II, while a larger number of the more chromogenic colonies were found in the samples from Stations 2/II and 3/II. However, chromogenic colonies were also observed in Station 1/II samples.

Of the 1,905 isolates selected for identification, 14 bacterial genera were represented. Six of these, *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Flavobacterium*, *Pseudomonas*, and *Vibrio* were found in both the heterotrophs and hydrocarbonoclastic isolates (Table 8.7).

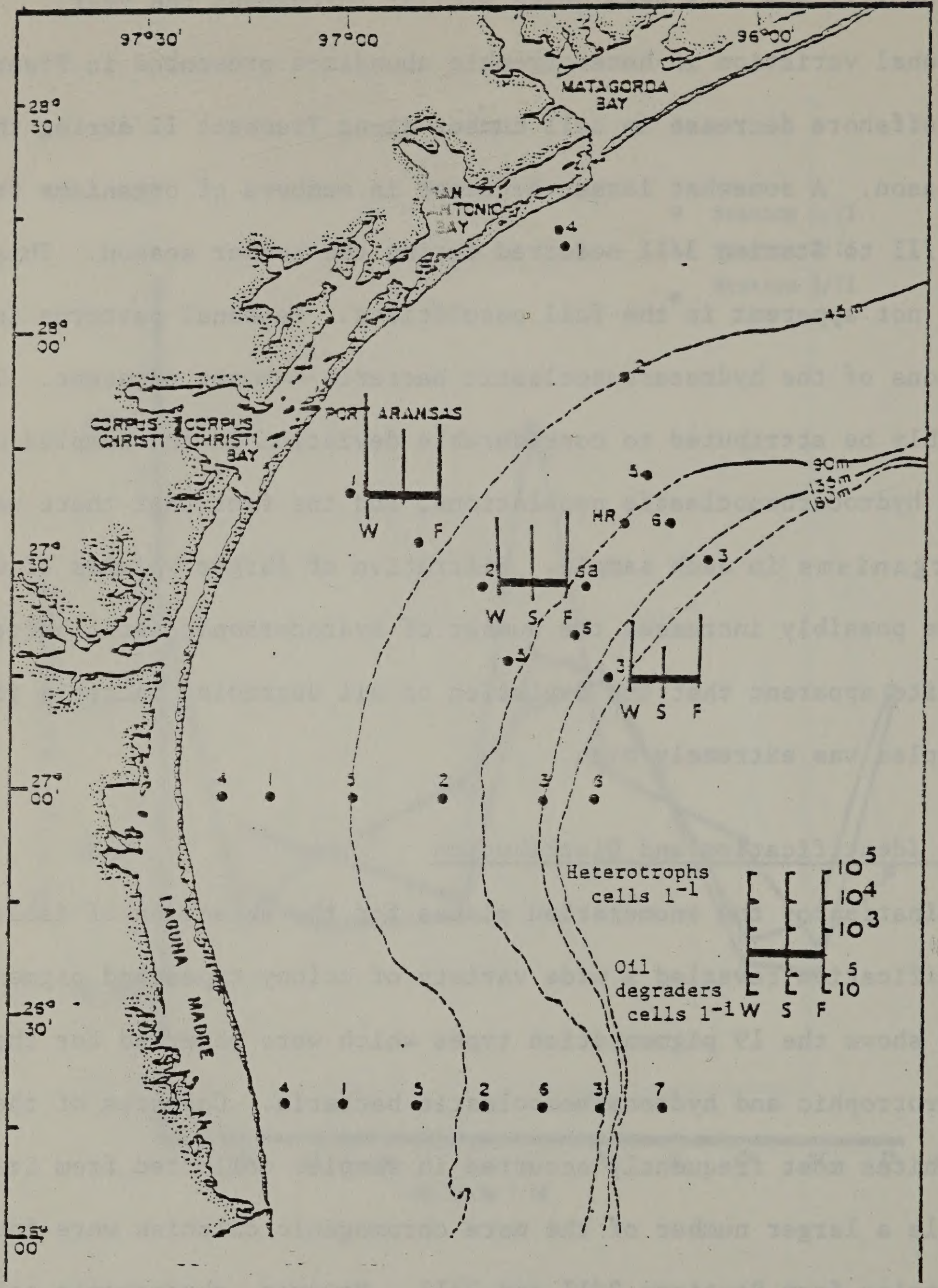


Figure 8.10 Seasonal Heterotrophic and Hydrocarbonoclastic (oil Degrader) Bacteria Abundance Variation in the Water Column Along Transect II (Depth 1 Meter).

TABLE 8.6

PIGMENTATION OF BACTERIA ISOLATED FROM WATER COLUMN SAMPLES COLLECTED AT STATIONS 1, 2, AND 3, TRANSECT II, IN 1977.

Pigmentation	Heterotroph*	Hydrocarbonoclastic**
Beige	X	X
Black	X	
Bluish Translucent	X	
Brown (Light)	X	X
Burgundy (Light)	X	
Cream	X	X
Dark Center	X	
Orange	X	X
Reddish Orange	X	X
Peach	X	
Pink		X
Pink Orange		X
Yellow	X	X
Yellow, Fluorescent Canary	X	
Red-Brown (Light)	X	
Red-Orange	X	X
White	X	X
White Translucent	X	X
Off White	X	X

* Isolated from samples plated on Marine Agar 2216.

** Isolated from samples plated on Silica Gel Oil, or Silica Gel Oil with fungizone.

TABLE 8.7

BACTERIAL GENERA OCCURRING IN WATER COLUMN SAMPLES COLLECTED ALONG TRANSECT II DURING 1977.

Genus	Heterotroph	Hydrocarbonoclastic
Acinetobacter	X	X
Aeromonas	X	X
Alcaligenes	X	X
Alteromonas	X	
Bacillus	X	
Enterobacteriaceae	X	
Flavobacterium	X	X
Moraxella	X	
Neisseria	X	
Photobacterium	X	
Planococcus	X	
Pseudomonas	X	X
Vibrio	X	X
Xanthomonas	X	

Tables 8.8 and 8.9 show the frequency distribution of the heterotrophic and hydrocarbonoclastic bacterial genera. *Pseudomonas* was the only genus represented in each of the 27 water samples. With regard to the heterotrophs, *Alcaligenes* did not occur at all in April, but did occur in 22 of the 27 samples collected. *Aeromonas* and *Vibrio* also occurred with great frequencies, 24 of 27 samples and 20 of 27 samples, respectively. With the hydrocarbonoclastic bacteria, *Pseudomonas* again was the predominant genus, followed by *Alcaligenes*, *Aeromonas*, and then *Vibrio*. The *Vibrio* genus occurred in only four of the 27 samples in the case of the hydrocarbonoclastic bacteria, and these samples were collected in April, June and July.

Tables 8.10, 8.11 and 8.12 show the seasonal distribution of heterotrophic genera. *Pseudomonas* appeared to be one of the predominants, if not the dominant, at all stations in each of the seasons. *Vibrio* isolates occurred most frequently at Station 1/II. The only luminescent genus isolated, the *Photobacterium*, was isolated only in April and June. Table 8.11 presents data supporting the fact that the greatest diversity in heterotrophs occurred in June and July, with the predominants being *Pseudomonas*, *Alcaligenes*, *Aeromonas*, and *Vibrio*.

Figure 8.11 shows the monthly variations of the four predominant genera in this year's study, *Pseudomonas*, *Alcaligenes*, *Aeromonas*, and *Vibrio*. The *Pseudomonas* population at Station 1/II exhibited large biphasic peaks in populations from April to June and again from August through November. All four organisms showed extremely fluctuating populations at Station 3/II. The *Pseudomonas*, *Aeromonas*, and *Vibrio* populations peaked at different times during the year at Station 2/II, which appeared to be the most stable of the three stations sampled.

The monthly fluctuations in the heterotrophic *Pseudomonas* populations

TABLE 8.8

FREQUENCY DISTRIBUTION OF HETEROTROPHIC BACTERIAL GENERA IN WATER COLUMN SAMPLES COLLECTED ALONG TRANSECT II

GENUS Month	FEB			MAR			APR			JUNE			JULY			AUG			OCT			NOV			DEC		
Station	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Acinetobacter		x	x						x		x	x	x	x		x		x	x					x	x	x	
Aeromonas	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x
Alcaligenes	x	x	x	x	x	x				x	x	x	x	x		x	x	x	x	x	x	x	x	x	x		x
Alteromonas																											x
Bacillus							x																				
Enterobacteriaceae											x										x						
Flavobacterium									x	x			x														
Moraxella												x															
Neisseria									x																		
Photobacterium								x	x	x																	
Planococcus													x														
Pseudomonas	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Vibrio	x			x	x		x	x	x	x	x	x		x	x		x		x		x	x	x	x	x	x	x
Xanthomonas									x																		

TABLE 8.9

FREQUENCY DISTRIBUTION OF HYDROCARBONOCLASTIC BACTERIAL GENERA IN WATER COLUMN SAMPLES COLLECTED ALONG TRANSECT II DURING 1977*

Genera	Month	Feb			Mar			Apr			Jun			July			Aug			Oct			Nov			Dec		
	Station	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Acinetobacter														X	X										X	X		
Aeromonas					X				X			X	X	X	X	X							X					
Alcaligenes					X			X	X		X	X			X	X	X	X	X	X	X	X	X			X	X	
Flavobacterium																					X			X				
Pseudomonas				X	X				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vibrio									X			X	X	X														

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* All hydrocarbonoclastic bacteria were isolated from cultures on Silica Gel Oil medium or Silica Gel Oil medium with fungizone.

TABLE 8.10

GENERA OF HETEROTROPHIC BACTERIA ISOLATED IN WATER COLUMN SAMPLES COLLECTED IN WINTER 1977 ON TRANSECT II

Genera	Station 1/II %	Station 2/II %	Station 3/II %
February:			
Acinetobacter	0.00	8.33 (4)*	13.33 (2)
Aeromonas	8.70 (6)	0.00	0.00
Alcaligenes	2.90 (2)	25.00 (12)	33.33 (5)
Pseudomonas	86.96 (60)	64.59 (31)	33.33 (5)
Vibrio	1.44 (1)	0.00	0.00
Unidentified		2.08 (1)	20.00 (3)
Viability Lost**	(1)	(12)	(25)
March:			
Aeromonas	22.22 (4)	16.67 (7)	30.77 (8)
Alcaligenes	38.89 (7)	9.72 (4)	7.69 (2)
Pseudomonas	11.11 (2)	21.43 (9)	61.54 (16)
Vibrio	11.11 (2)	42.86 (18)	0.00
Unidentified	16.67 (3)	9.25 (4)	
Viability Lost**	(1)	(3)	
April:			
Acinetobacter	0.00	0.00	2.00 (1)
Aeromonas	18.31 (13)	14.28 (4)	28.00 (14)
Bacillus	1.41 (1)	0.00	0.00
Flavobacterium	0.00	0.00	2.00 (1)
Neisseria	0.00	0.00	2.00 (1)
Photobacterium	0.00	28.57 (8)	2.00 (1)
Pseudomonas	52.11 (37)	17.86 (5)	16.00 (8)
Vibrio	28.17 (20)	39.29 (11)	46.00 (23)
Xanthomonas	0.00	0.00	2.00 (1)
Viability Lost**		(4)	(2)

---

* Number in parenthesis indicates number of isolates.

** Isolates which lost their viability prior to completion of the microscopic and biochemical tests for identification.

TABLE 8.11

GENERA OF HETEROTROPHIC BACTERIA ISOLATED IN WATER COLUMN SAMPLES COLLECTED IN SUMMER 1977 ON TRANSECT II.

Genera	Station 1/II %	Station 2/II %	Station 3/II %
June:			
Acinetobacter	0.00	2.44 (3)*	8.41 (9)
Aeromonas	9.33 (18)	4.07 (5)	9.35 (10)
Alcaligenes	8.29 (16)	4.88 (6)	14.95 (16)
Enterobacteriaceae	0.00	0.81 (1)	0.00
Flavobacterium	0.52 (1)	0.00	0.00
Moraxella	0.00	0.00	0.94 (1)
Photobacterium	1.04 (2)	0.00	0.00
Pseudomonas	55.95 (108)	22.76 (28)	61.68 (66)
Vibrio	23.83 (46)	65.04 (80)	2.80 (3)
Unidentified	1.04 (2)	0.00	1.87 (2)
Viability lost**	(2)	(2)	(7)
July:			
Acinetobacter	5.66 (3)	4.76 (2)	0.00
Aeromonas	18.87 (10)	19.05 (8)	59.38 (19)
Alcaligenes	32.07 (17)	19.05 (8)	0.00
Flavobacterium	7.54 (4)	0.00	0.00
Planococcus	1.89 (1)	0.00	0.00
Pseudomonas	32.07 (17)	35.71 (15)	3.12 (1)
Vibrio	0.00	21.43 (9)	37.50 (12)
Unidentified	1.89 (1)	0.00	0.00
Viability lost**	(3)	(0)	(21)
August:			
Acinetobacter	1.82 (1)	0.00	12.25 (6)
Aeromonas	34.54 (19)	3.57 (2)	4.08 (2)
Alcaligenes	3.64 (2)	25.00 (14)	4.08 (2)
Pseudomonas	60.00 (33)	57.14 (32)	79.59 (39)
Vibrio	0.00	14.29 (8)	0.00
Viability lost**	(1)	(0)	(2)

* Number in parenthesis indicates number of isolates classified.

** Isolates from original Marine Agar 2216 plates which lost their viability prior to completion of the microscopic and physiological tests. These were not included in the calculation of percentages for the genera shown.

TABLE 8.12

GENERA OF HETEROTROPHIC BACTERIA ISOLATED IN WATER COLUMN SAMPLES COLLECTED IN FALL 1977 ALONG TRANSECT II.

Genera	Station 1/II %	Station 2/II %	Station 3/II %
October:			
Acinetobacter	3.85 (2)*	0.00	0.00
Aeromonas	9.61 (5)	46.51 (20)	46.15 (12)
Alcaligenes	5.77 (3)	13.95 (6)	3.85 (1)
Enterobacteriaceae	0.00	0.00	3.85 (1)
Pseudomonas	78.85 (41)	39.54 (17)	11.54 (3)
Vibrio	1.92 (1)	0.00	19.23 (5)
Unidentified			15.38 (4)
Viability Lost**	(0)	(2)	(0)
November:			
Acinetobacter	0.00	0.00	5.72 (2)
Aeromonas	0.00	51.61 (16)	20.00 (7)
Alcaligenes	16.67 (5)	29.03 (9)	20.00 (7)
Pseudomonas	70.00 (21)	12.91 (4)	25.71 (9)
Vibrio	3.33 (1)	6.45 (2)	8.57 (3)
Unidentified	10.00 (3)		20.00 (7)
Viability Lost**	(11)	(0)	(0)
December:			
Acinetobacter	25.00 (8)	3.23 (1)	0.00
Aeromonas	9.38 (3)	0.00	12.12 (4)
Alcaligenes	28.12 (9)	9.67 (3)	12.12 (4)
Alteromonas	0.00	0.00	3.03 (1)
Pseudomonas	31.25 (10)	51.61 (16)	63.64 (21)
Vibrio	6.25 (2)	32.26 (10)	9.09 (3)
Unidentified		3.23 (1)	
Viability Lost**	(0)	(0)	(0)

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* Number in parenthesis indicates number of bacterial isolates.

** Organisms losing viability prior to completion of the microscopic and physiological studies for identification.

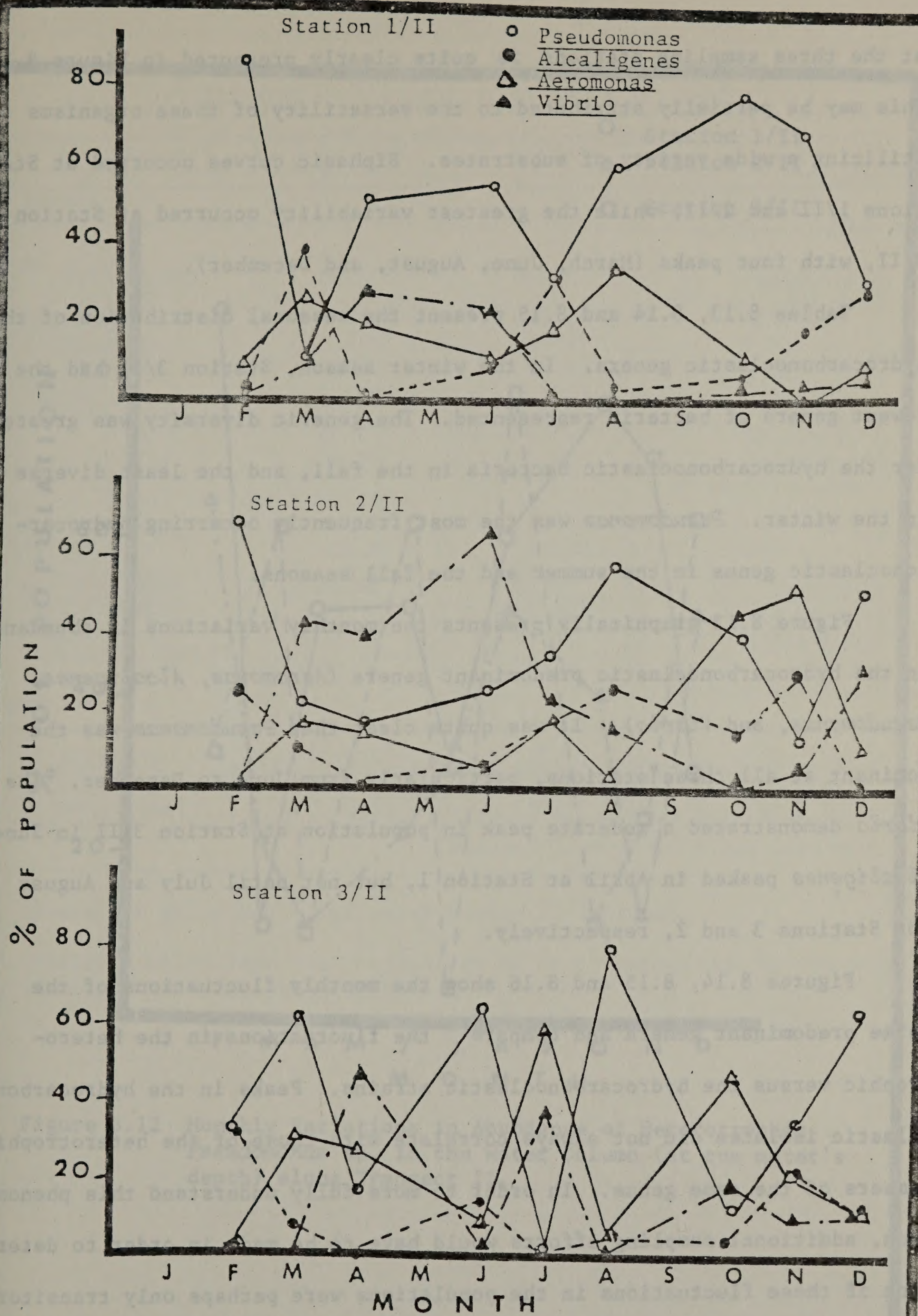


Figure 8.11 Monthly Variation in Abundance of Four Predominant Genera of Heterotrophic Bacteria in the Water Column Along Transect II.

at the three sampling stations is quite clearly presented in Figure 8.12. This may be partially attributed to the versatility of these organisms for utilizing a wide variety of substrates. Biphasic curves occurred at Stations 1/II and 2/II, while the greatest variability occurred at Station 3/II, with four peaks (March, June, August, and December).

Tables 8.13, 8.14 and 8.15 present the seasonal distribution of the hydrocarbonoclastic genera. In the winter season, Station 3/II had the fewest genera of bacteria represented. The generic diversity was greatest for the hydrocarbonoclastic bacteria in the fall, and the least diverse in the winter. *Pseudomonas* was the most frequently occurring hydrocarbonoclastic genus in the summer and the fall seasons.

Figure 8.13 graphically presents the monthly variations in abundance in the hydrocarbonoclastic predominant genera (*Aeromonas*, *Alcaligenes*, *Pseudomonas*, and *Vibrio*). It was quite clear that *Pseudomonas* was the dominant at all three stations, particularly from June to December. The *Vibrio* demonstrated a moderate peak in population at Station 3/II in June. *Alcaligenes* peaked in April at Station 1, but not until July and August for Stations 3 and 2, respectively.

Figures 8.14, 8.15 and 8.16 show the monthly fluctuations of the three predominant genera and compare the fluctuations in the heterotrophic versus the hydrocarbonoclastic strains. Peaks in the hydrocarbonoclastic isolates did not always correlate with those of the heterotrophic members of the same genus. In order to more fully understand this phenomenon, additional sampling efforts would have to be made in order to determine if these fluctuations in the populations were perhaps only transitory, rather than normal population variations. In general, the monthly variation of *Alcaligenes* were not as drastic as those of *Pseudomonas* and *Aeromonas*.

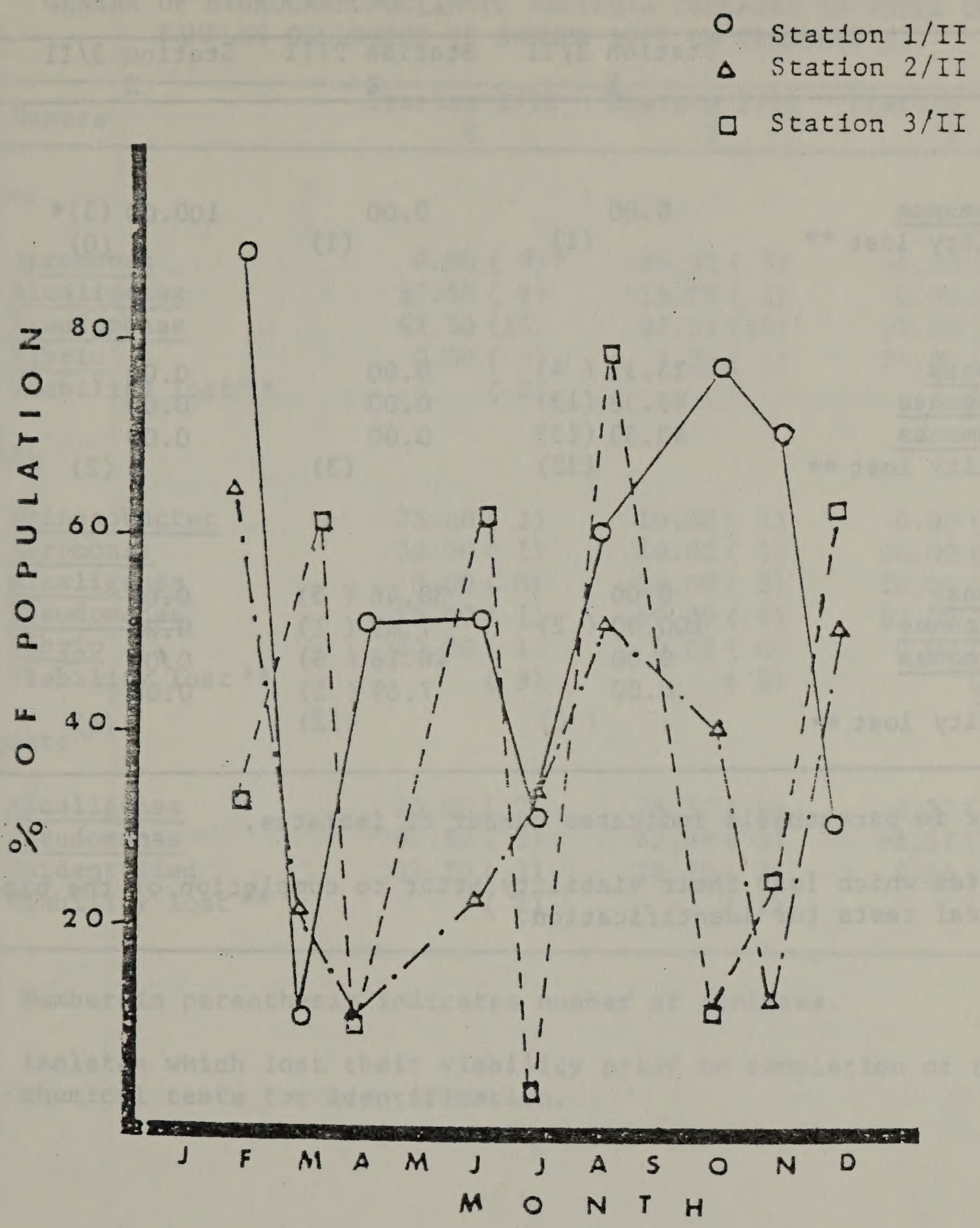


Figure 8.12 Monthly Variations in Abundance of Heterotrophic *Pseudomonas* sp. in the Water Column (at one meter's depth) along Transect II.

TABLE 8.13

GENERA OF HYDROCARBONOCLASTIC BACTERIA ISOLATED IN WATER COLUMN SAMPLES COLLECTED IN WINTER 1977 ON TRANSECT II.

Genera	Station 1/II %	Station 2/II %	Station 3/II %
February:			
Pseudomonas	0.00	0.00	100.00 (3)*
Viability lost **	(1)	(1)	(0)
March:			
Aeromonas	13.33 (4)	0.00	0.00
Alcaligenes	43.33 (13)	0.00	0.00
Pseudomonas	43.33 (13)	0.00	0.00
Viability lost **	(22)	(3)	(2)
April:			
Aeromonas	0.00	38.46 (5)	0.00
Alcaligenes	100.00 (2)	7.69 (1)	0.00
Pseudomonas	0.00	46.16 (6)	0.00
Vibrio	0.00	7.69 (1)	0.00
Viability lost **	(0)	(12)	(6)

* Number in parenthesis indicates number of isolates.

** Isolates which lost their viability prior to completion of the biochemical tests for identification.

TABLE 8.14

GENERA OF HYDROCARBONOCLASTIC BACTERIA ISOLATED IN WATER COLUMN SAMPLES COLLECTED IN SUMMER 1977 ON TRANSECT II.

Genera	Station 1/II %	Station 2/II %	Station 3/II %
June:			
Aeromonas	0.00 (0)*	26.32 (5)	44.00 (11)
Alcaligenes	37.50 (9)	15.79 (3)	0.00 (0)
Pseudomonas	62.50 (15)	52.63 (10)	32.00 (8)
Vibrio	0.00 (0)	5.26 (1)	24.00 (6)
Viability lost **	(0)		(0)
July:			
Acinetobacter	25.00 (1)	10.00 (1)	0.00 (0)
Aeromonas	25.00 (1)	10.00 (1)	20.00 (1)
Alcaligenes	0.00 (0)	20.00 (2)	20.00 (1)
Pseudomonas	25.00 (1)	60.00 (6)	60.00 (3)
Vibrio	25.00 (1)	0.00 (0)	0.00 (0)
Viability lost **	(9)	(8)	(1)
August:			
Alcaligenes	25.00 (2)	28.57 (2)	8.57 (3)
Pseudomonas	37.50 (3)	42.86 (3)	88.57 (31)
Unidentified	37.50 (3)	28.57 (2)	2.86 (1)
Viability lost **	(2)	(1)	(9)

* Number in parenthesis indicates number of isolates.

** Isolates which lost their viability prior to completion of the biochemical tests for identification.

TABLE 8.15

GENERA OF HYDROCARBONOCLASTIC BACTERIA ISOLATED IN WATER COLUMN SAMPLES COLLECTED IN FALL 1977 ON TRANSECT II.

Genera	Station 1/II %	Station 2/II %	Station 3/II %
October:			
Alcaligenes	27.27 (3)*	28.57 (4)	16.67 (1)
Flavobacterium	0.00	7.14 (1)	0.00
Pseudomonas	63.64 (7)	57.15 (8)	83.33 (5)
Unidentified	9.09 (1)	7.14 (1)	
Viability lost **	(3)	(2)	(0)
November:			
Acinetobacter	0.00	0.00	6.67 (1)
Aeromonas	6.66 (1)	0.00	0.00
Alcaligenes	26.67 (4)	0.00	0.00
Flavobacterium	0.00	16.67 (1)	0.00
Pseudomonas	46.67 (7)	33.33 (2)	40.00 (6)
Unidentified	20.00 (3)	50.00 (3)	53.33 (8)
Viability lost **	(5)	(7)	(11)
December:			
Acinetobacter	3.57 (1)		
Alcaligenes	10.72 (3)	19.05 (4)	
Pseudomonas	71.42 (20)	61.90 (13)	100.00 (1)
Unidentified	14.29 (4)	19.05 (4)	
Viability lost **	(8)	(1)	(0)

* Number in parenthesis indicates number of isolates.

** Isolates which lost their viability prior to completion of the biochemical tests for identification.

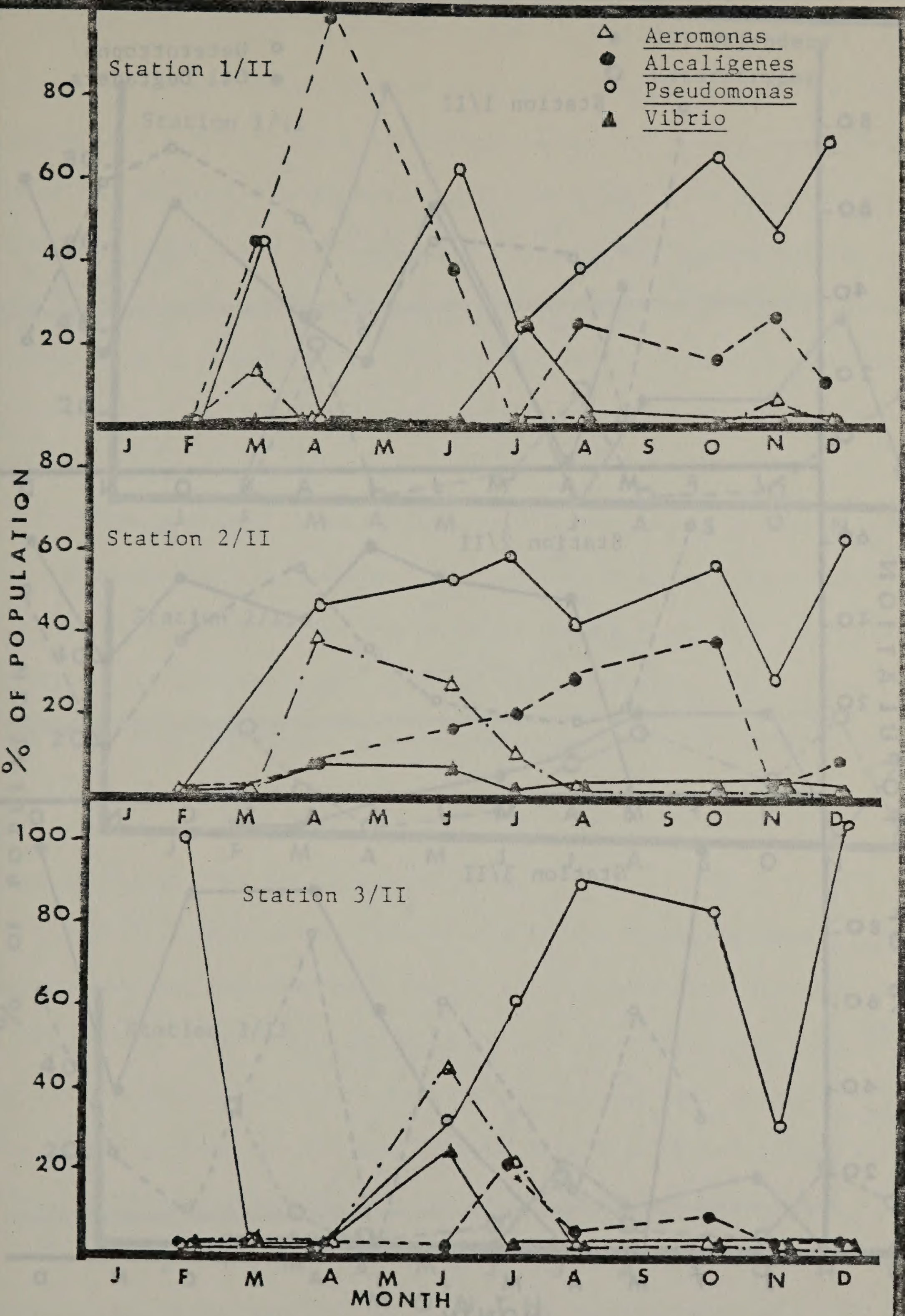


Figure 8.13 Monthly Variations in Abundance of Four Predominant Genera of Hydrocarbonoclastic Bacteria in the Water Column Along Transect II.

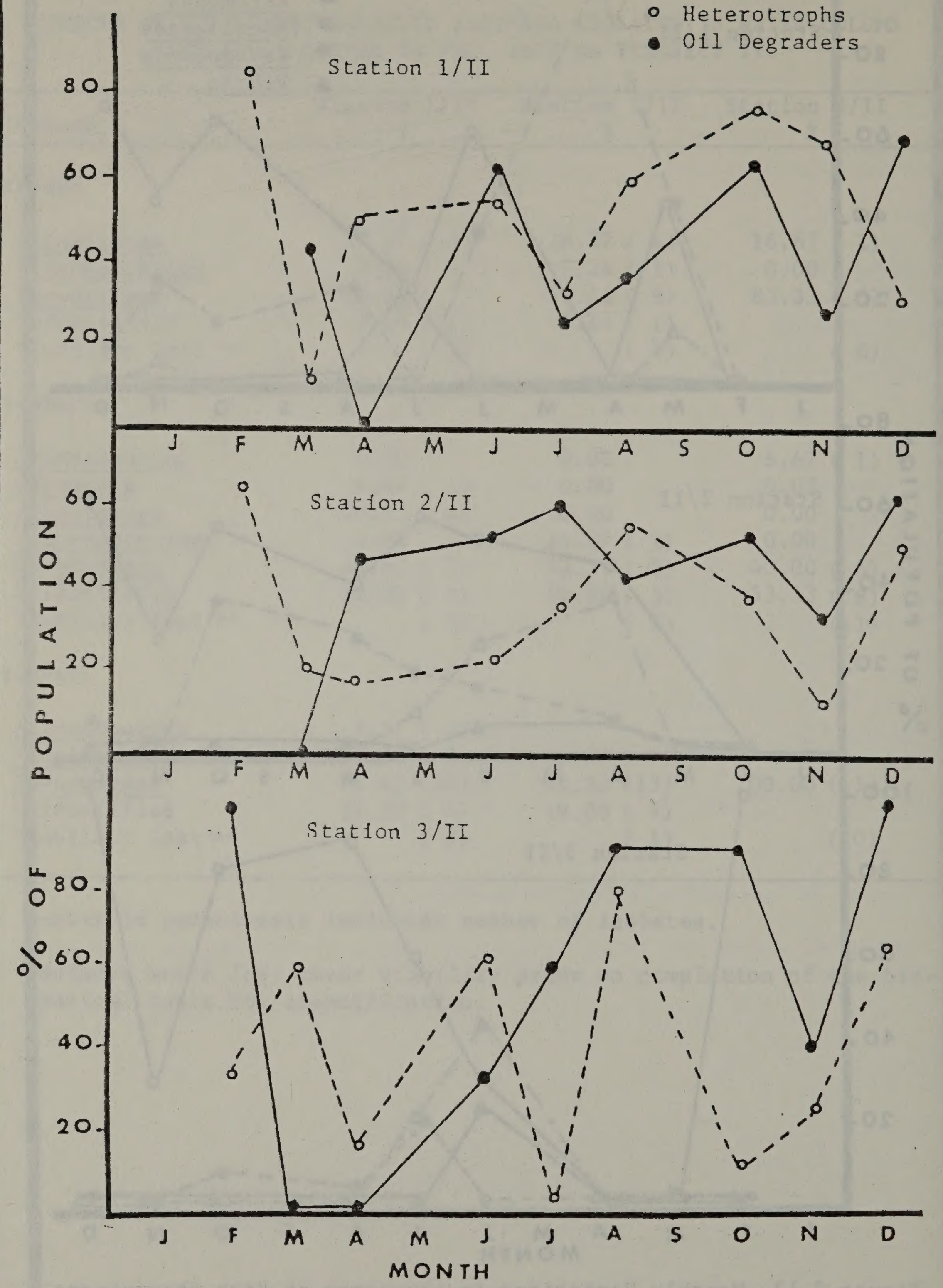


Figure 8.14 Monthly Variations in Abundance of Heterotrophic and Hydrocarbonoclastic (Oil Degrading) *Pseudomonas* sp. in the Water Column (One Meter's Depth) Along Transect II.

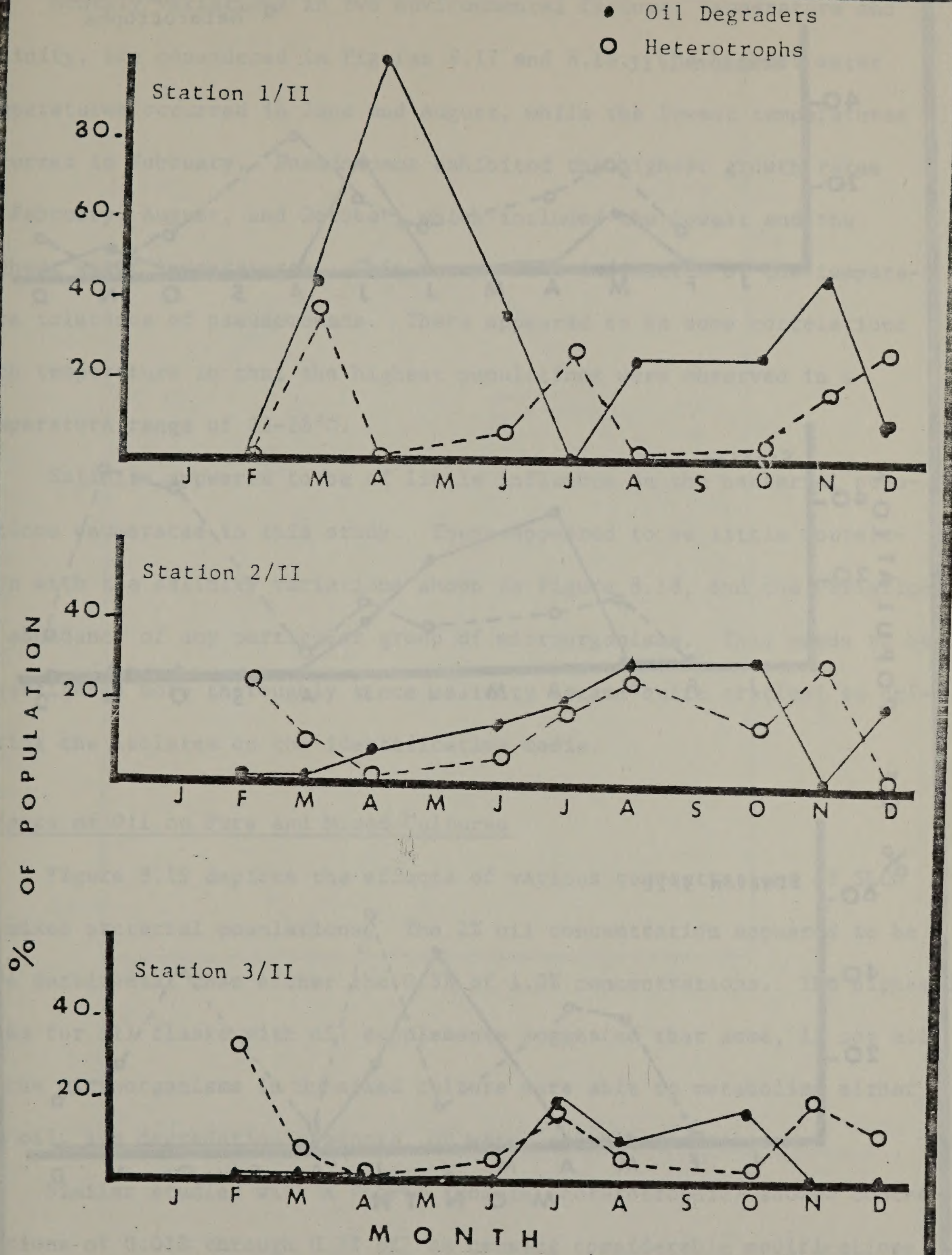


Figure 8.15 Monthly Variation in Abundance of Heterotrophic and Hydrocarbonoclastic (Oil Degrading) *Alcaligenes* sp. in the Water Column (One Meter's Depth) Along Transect II.

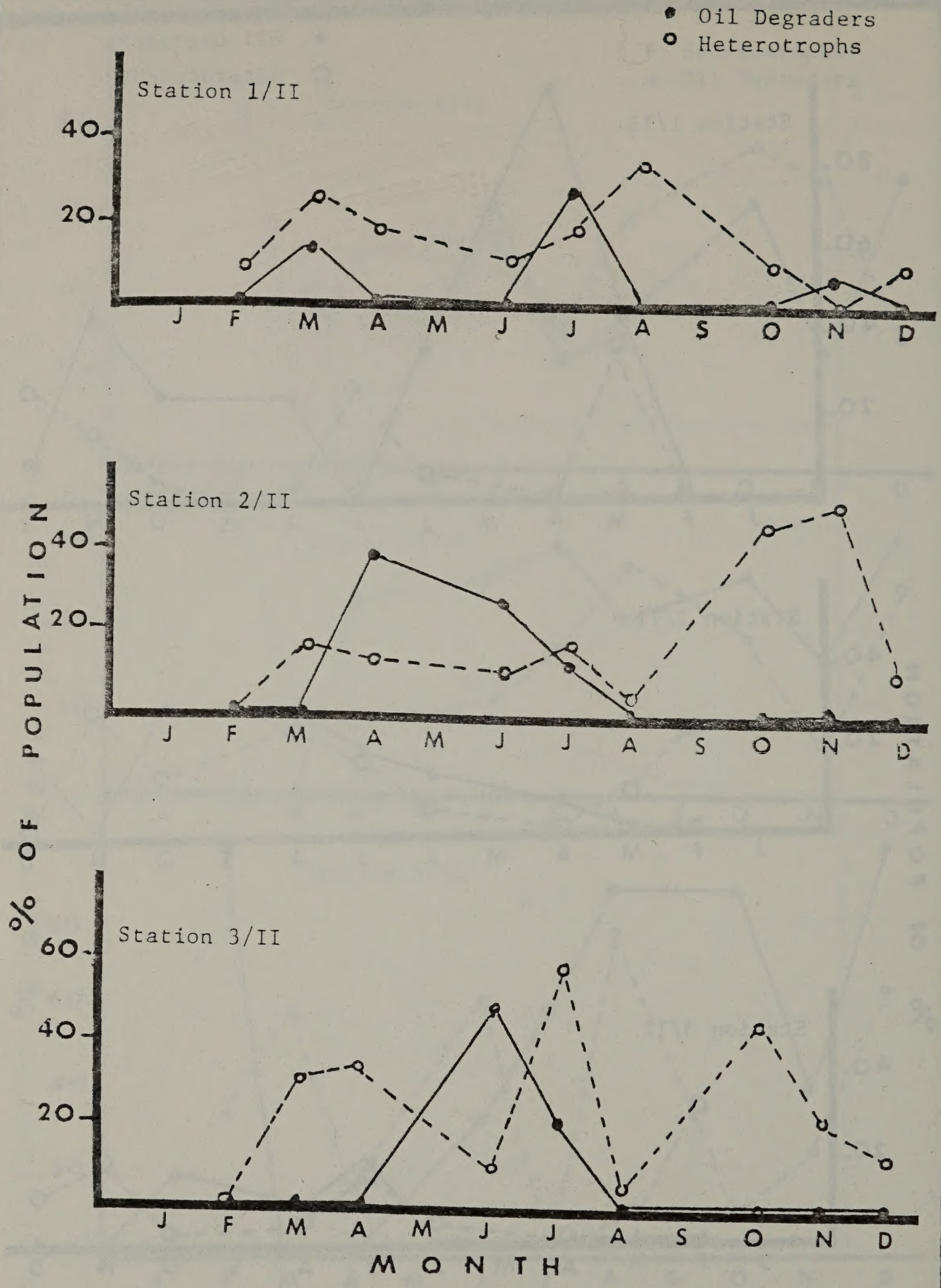


Figure 8.16 Monthly Variation in the Abundance of Heterotrophic and Hydrocarbonoclastic (Oil Degrading) *Aeromonas* sp. in the Water Column (One Meter's Depth) Along Transect II.

Monthly variations in two environmental factors, temperature and salinity, are considered in Figures 8.17 and 8.18. The highest water temperatures occurred in June and August, while the lowest temperatures occurred in February. *Pseudomonas* exhibited the highest growth rates in February, August, and October, which included the lowest and the highest water temperatures. This was a clear indication of the temperature tolerance of pseudomonads. There appeared to be some correlations with temperature in that the highest populations were observed in a temperature range of 23-26°C.

Salinity appeared to be of little influence on the bacterial populations enumerated in this study. There appeared to be little correlation with the salinity variations shown in Figure 8.18, and the variations in abundance of any particular group of microorganisms. This needs to be investigated more thoroughly since salinity became quite critical in culturing the isolates on the identification media.

Effects of Oil on Pure and Mixed Cultures

Figure 8.19 depicts the effects of various concentrations of SLCO on mixed bacterial populations. The 2% oil concentration appeared to be more detrimental than either the 0.5% of 1.0% concentrations. The biphasic peaks for all flasks with oil supplements suggested that some, if not all, of the microorganisms in the mixed culture were able to metabolize either the oil, its degradation products, or water soluble fractions.

Similar studies with a *Vibrio* isolate (heterotrophic) showed concentrations of 0.01% through 0.1% oil as causing considerable modifications in the growth rate of the organism. This is clearly shown in Figure 8.20. Nutrition studies with this isolate showed that the organism required supplements of peptone for a carbon and a nitrogen source to have optimum growth. Apparently, it was able to utilize some of the lower concentrations

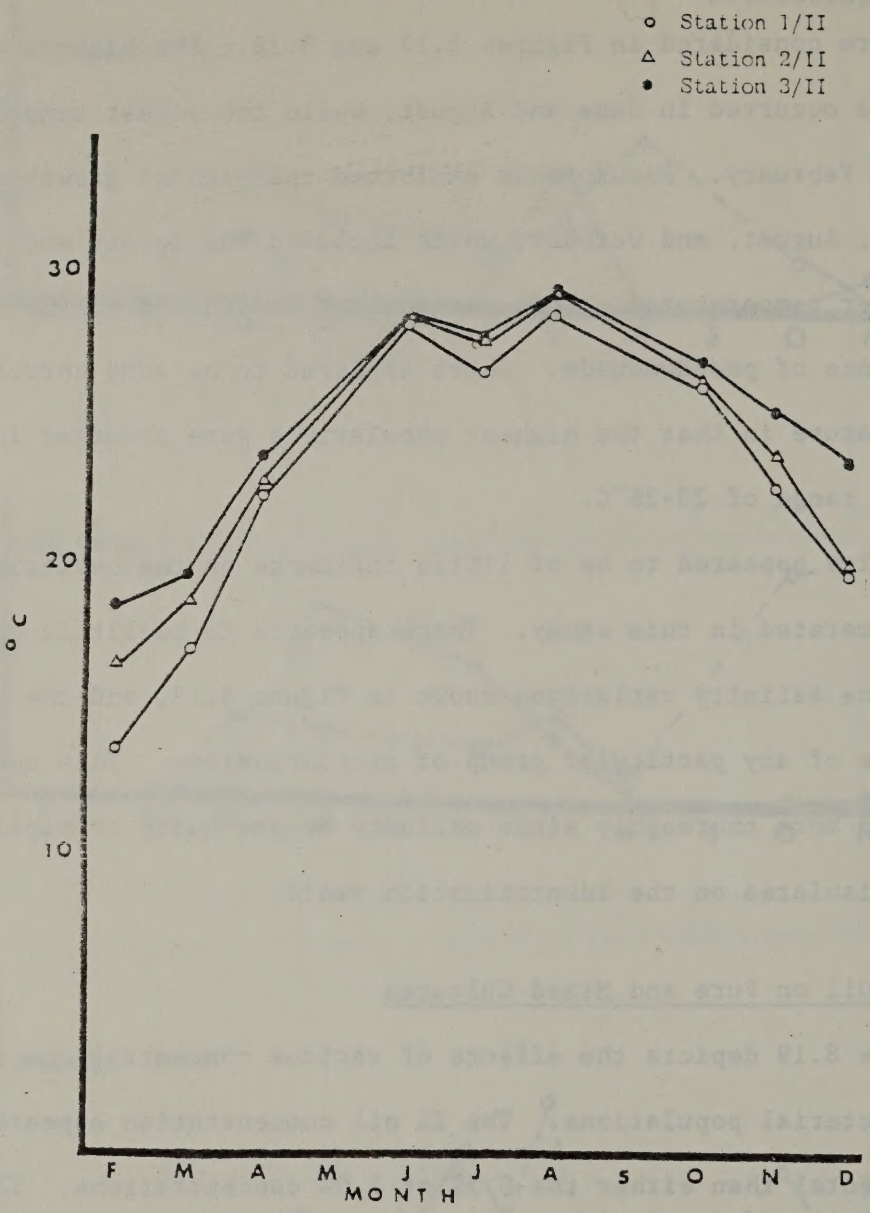


Figure 8.17 Monthly Variations of Surface Water Temperature Along Transect II at the Time of Collection of Water Column Samples for the Bacteriological Studies.

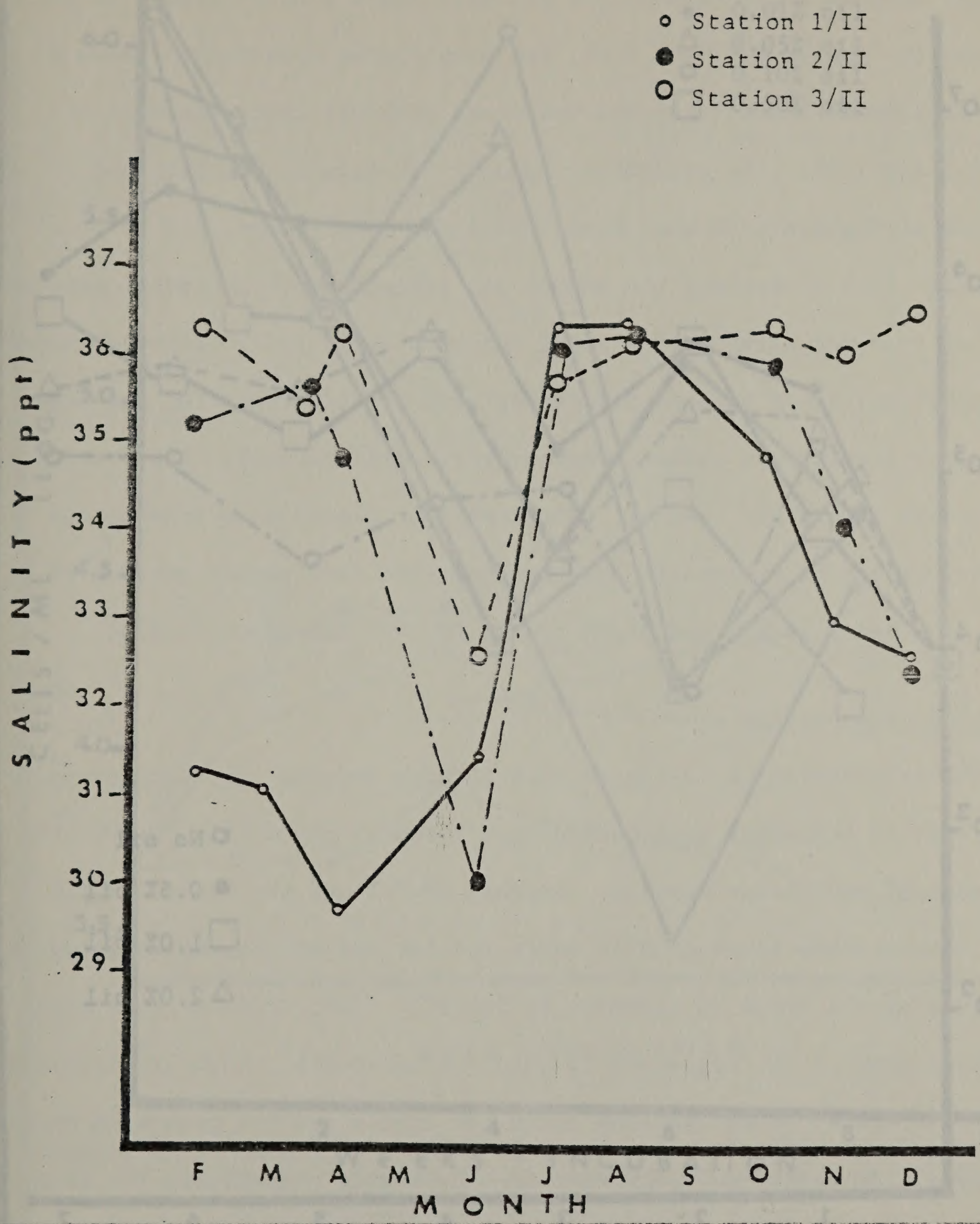


Figure 8.18 Monthly Variations in Salinity (ppt) of the Water Column (One Meter's Depth) Along Transect II.

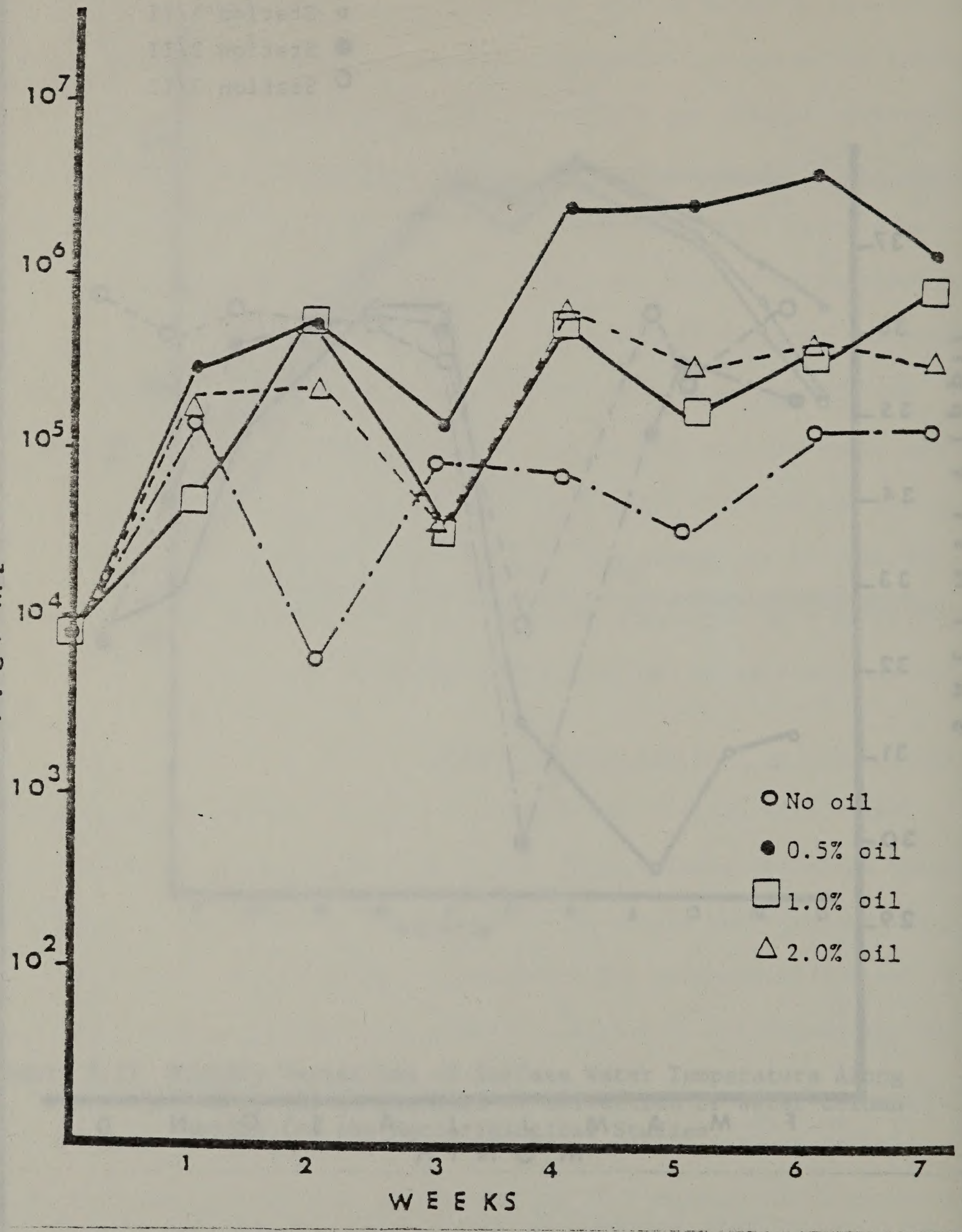


Figure 8.19 Effects of Various Concentrations of South Louisiana Crude Oil on Mixed Bacterial Populations from Station 1/II Samples of August 1977.

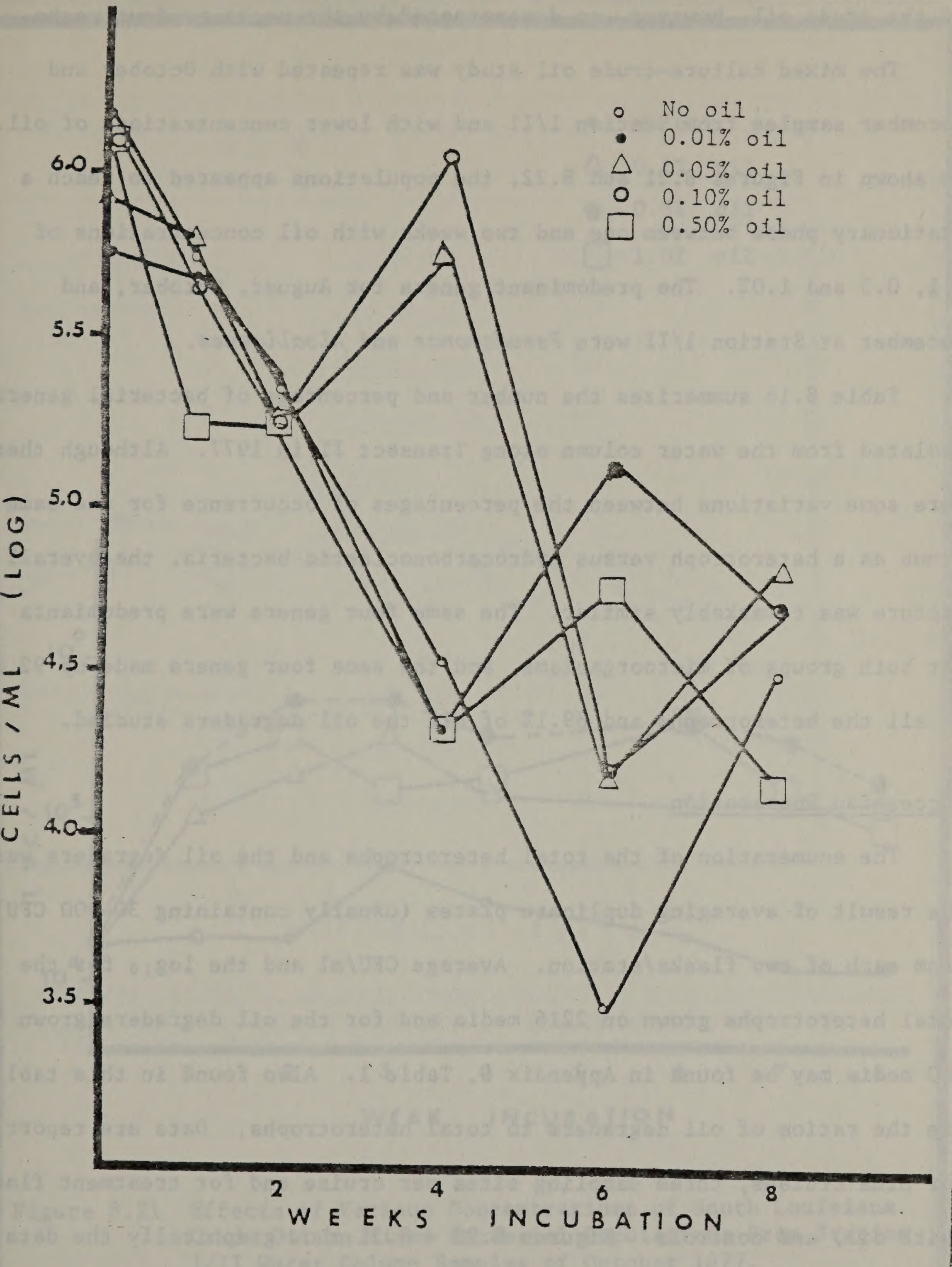


Figure 8.20 Effects of Various Concentrations of South Lousiana Crude Oil on the Growth Rate of *Vibrio* Isolate of Station 3/II.

of the crude oil, however, as demonstrated by the peaks at four weeks.

The mixed culture-crude oil study was repeated with October and December samples from Station 1/II and with lower concentrations of oil. As shown in Figures 8.21 and 8.22, the populations appeared to reach a stationary phase between one and two weeks with oil concentrations of 0.1, 0.5 and 1.0%. The predominant genera for August, October, and December at Station 1/II were *Pseudomonas* and *Alcaligenes*.

Table 8.16 summarizes the number and percentage of bacterial genera isolated from the water column along Transect II in 1977. Although there were some variations between the percentages of occurrence for the same genus as a heterotroph versus hydrocarbonoclastic bacteria, the overall picture was remarkably similar. The same four genera were predomiants for both groups of microorganisms, and the same four genera made up 92.8% of all the heterotrophs and 89.1% of all the oil degraders studied.

Succession Enumeration

The enumeration of the total heterotrophs and the oil degraders was the result of averaging duplicate plates (usually containing 30-300 CFU) from each of two flasks/station. Average CFU/ml and the $\log_{10}$ for the total heterotrophs grown on 2216 media and for the oil degraders grown on SGO media may be found in Appendix G, Table 1. Also found in this table are the ratios of oil degraders to total heterotrophs. Data are reported for nine cruises, three sampling sites per cruise and for treatment flasks (with oil) and controls. Figures 8.23 - 8.31 show graphically the data represented in the above mentioned tables. Note in these figures that both heterotrophs and oil degraders reached a relatively steady state population in a relatively short time and then did not deviate greatly. The August cruise (Figure 8.28) was the best example of this rapid increase in

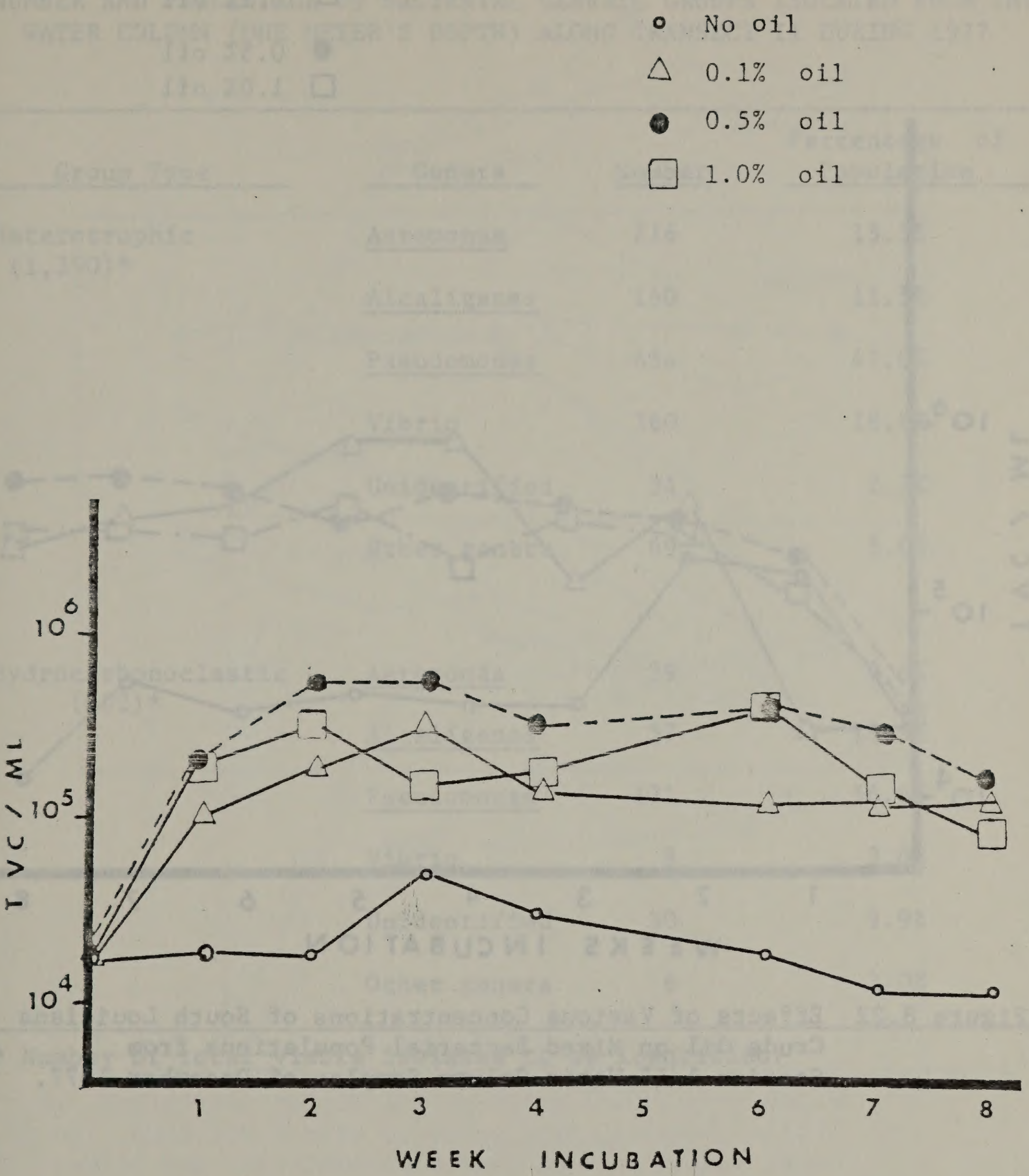


Figure 8.21 Effects of Various Concentrations of South Louisiana Crude Oil on Mixed Bacterial Populations From Station 1/II Water Column Samples of October 1977.

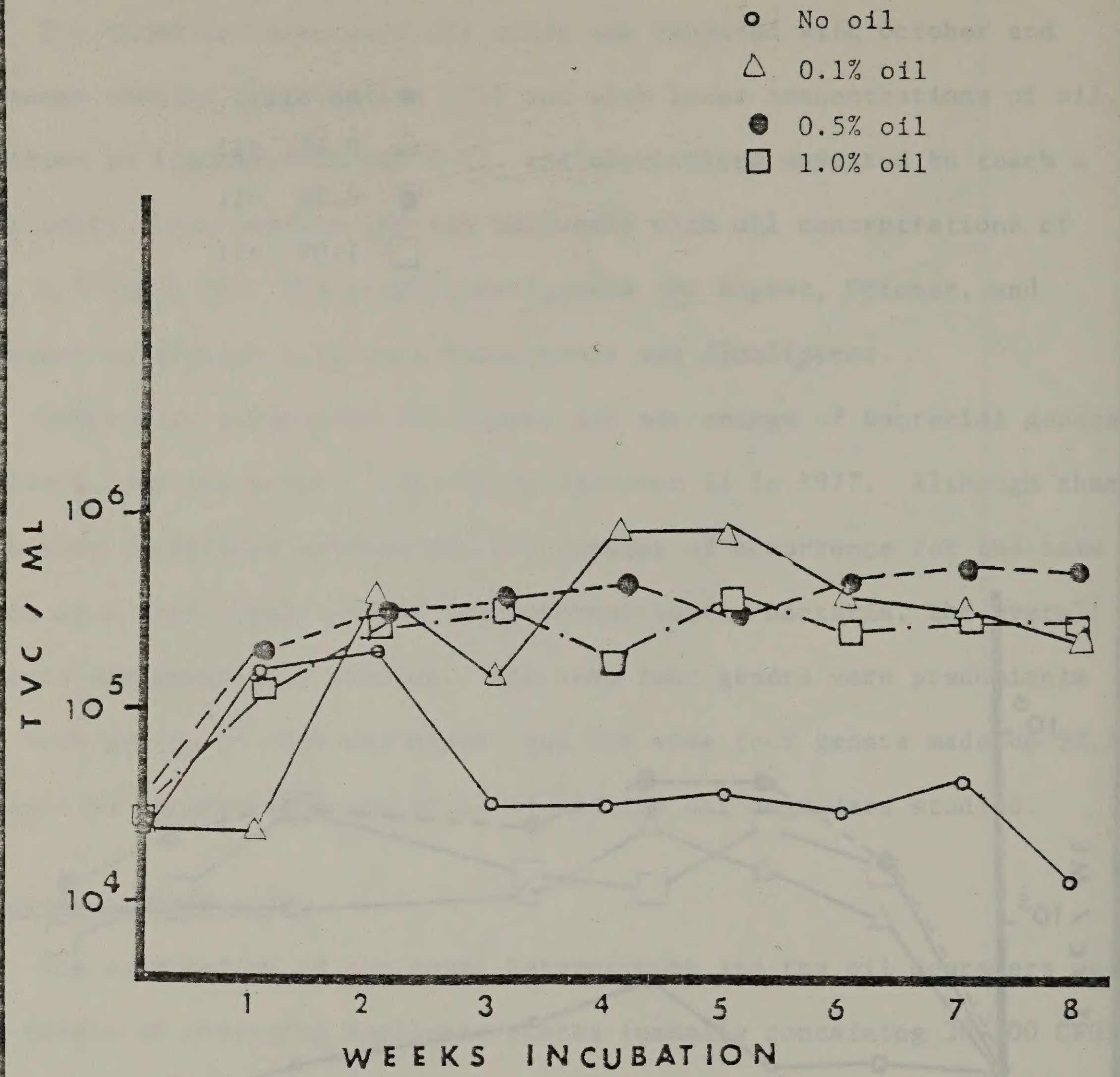


Figure 8.22 Effects of Various Concentrations of South Louisiana Crude Oil on Mixed Bacterial Populations from Station 1/II Water Column Samples of December 1977.

TABLE 8.16

NUMBER AND PERCENTAGE OF BACTERIAL GENERIC GROUPS ISOLATED FROM THE WATER COLUMN (ONE METER'S DEPTH) ALONG TRANSECT II DURING 1977

Group Type	Genera	Number	Percentage of Population
Heterotrophic (1,390)*	Aeromonas	216	15.5%
	Alcaligenes	160	11.5%
	Pseudomonas	654	47.0%
	Vibrio	260	18.8%
	Unidentified	31	2.2%
	Other genera	69	5.0%
Hydrocarbonoclastic (302)*	Aeromonas	29	9.6%
	Alcaligenes	57	18.9%
	Pseudomonas	171	56.6%
	Vibrio	9	3.0%
	Unidentified	30	9.9%
	Other genera	6	2.0%

* Number of total viable isolates to be identified.

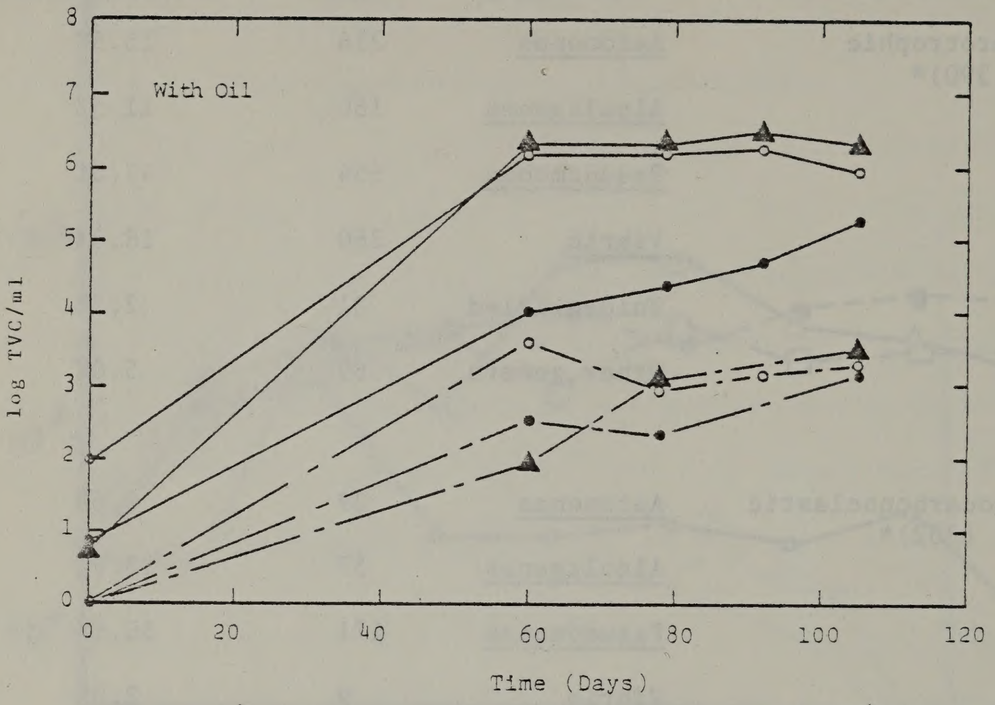


Figure 8.23 Microbial Populations (TVC= total viable counts) in Water Samples Collected on the Winter Cruise(Feb. 17, 1977). Samples were Incubated with 0.05% SLCO. Solid Lines Indicate Total Marine Heterotrophs and Broken Lines Indicate Oil Degraders.

○ = Station 1; ● = Station 2; ▲= Station 3.

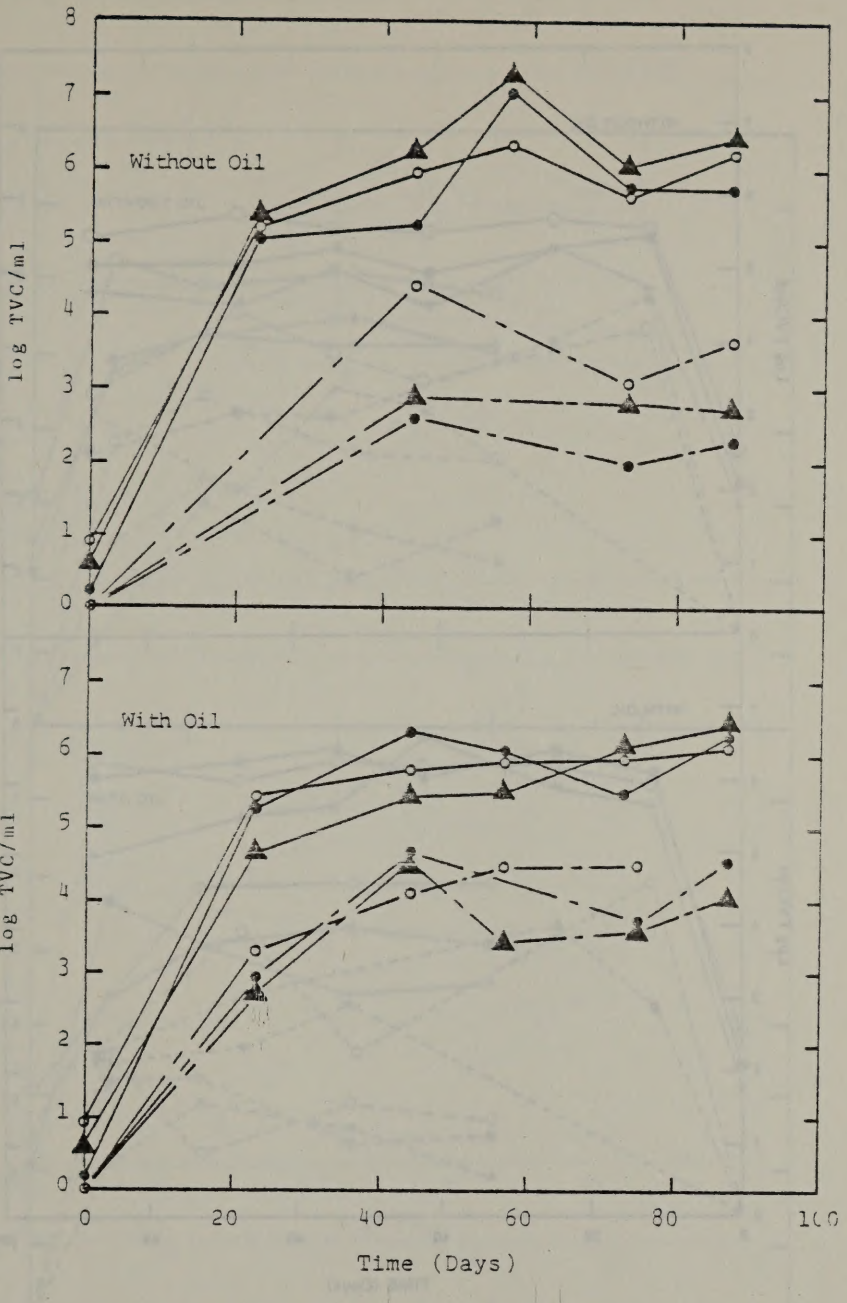


Figure 8.24 Microbial Populations (TVC = total viable counts) in Water Samples Collected on the March cruise (March 25). Samples were Incubated With or Without 0.5% SLCO. Solid Lines Indicate Total Marine Heterotrophs and Broken Lines Indicate Oil Degraders.
○ = Station 1; ● = Station 2; ▲ = Station 3.

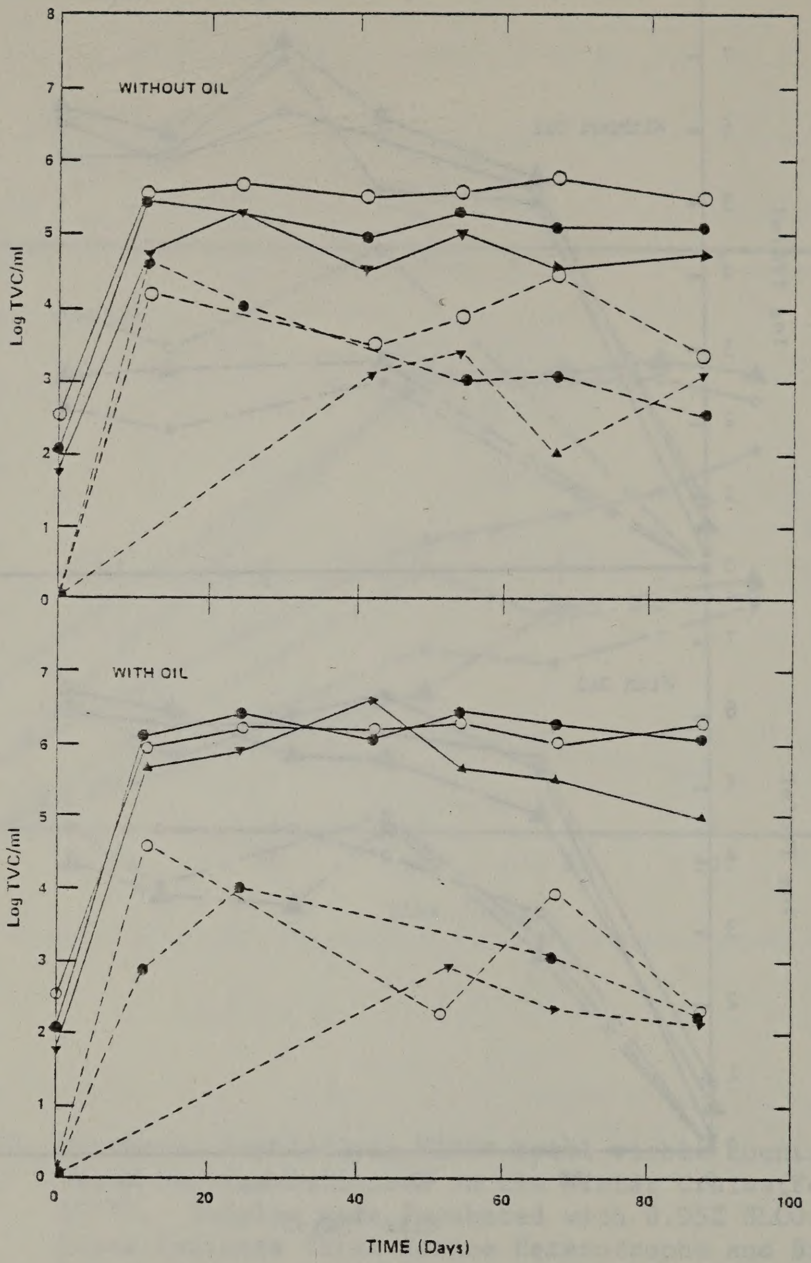


Figure 8.25 Microbial Populations (TVC = total viable counts) in Water Samples Collected on the April Cruise (April 25). Samples were Incubated With or Without 0.5% SLCO. Solid Lines Indicate Total Marine Heterotrophs and Broken Lines Indicate Oil Degraders.
○ = Station 1; ● = Station 2; ▲ = Station 3

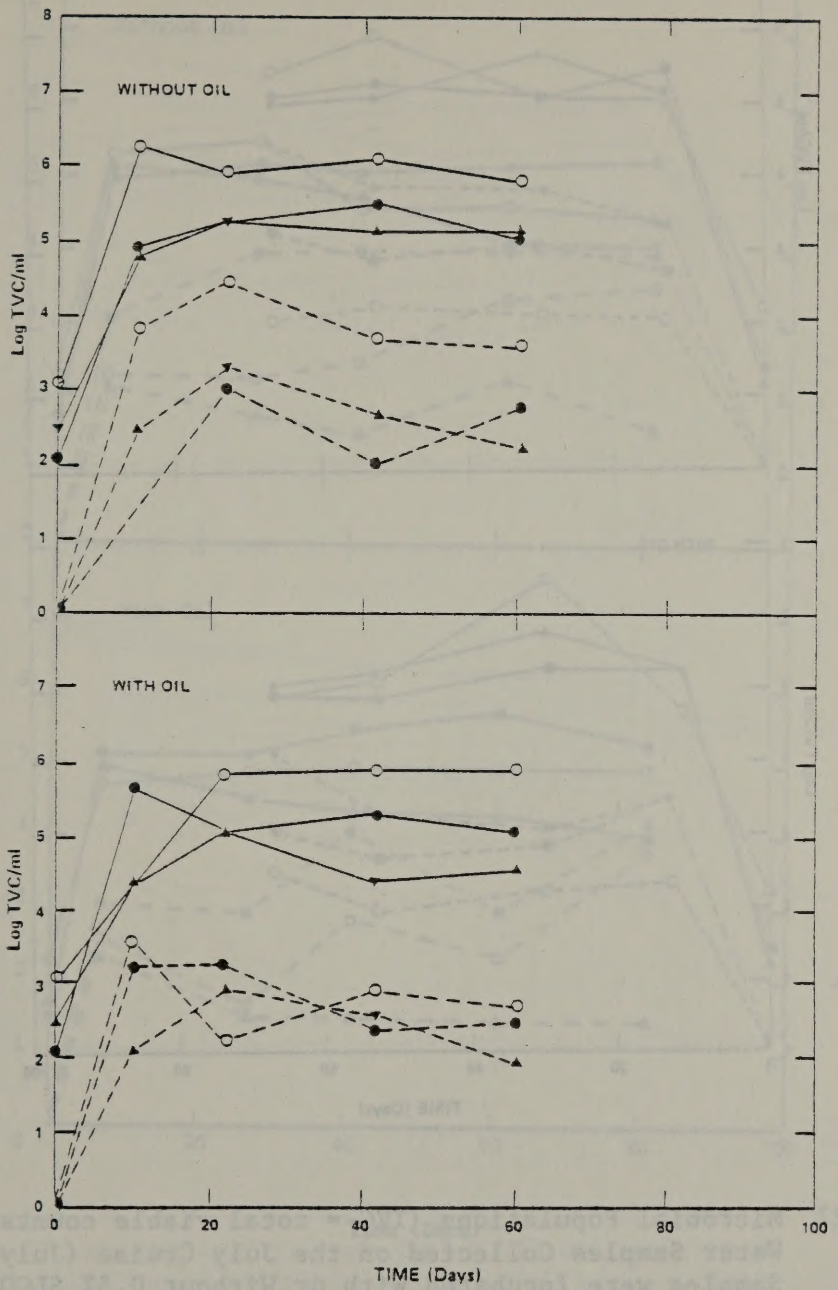


Figure 8.26 Microbial Populations (TVC = total viable counts) in Water Samples Collected on the Spring Cruise (June 10). Samples were Incubated With or Without 0.5% SLCO. Solid Lines Indicate Total Marine Heterotrophs and Broken Lines Indicate Oil Degraders.

○ = Station 1; ● = Station 2; ▲ = Station 3

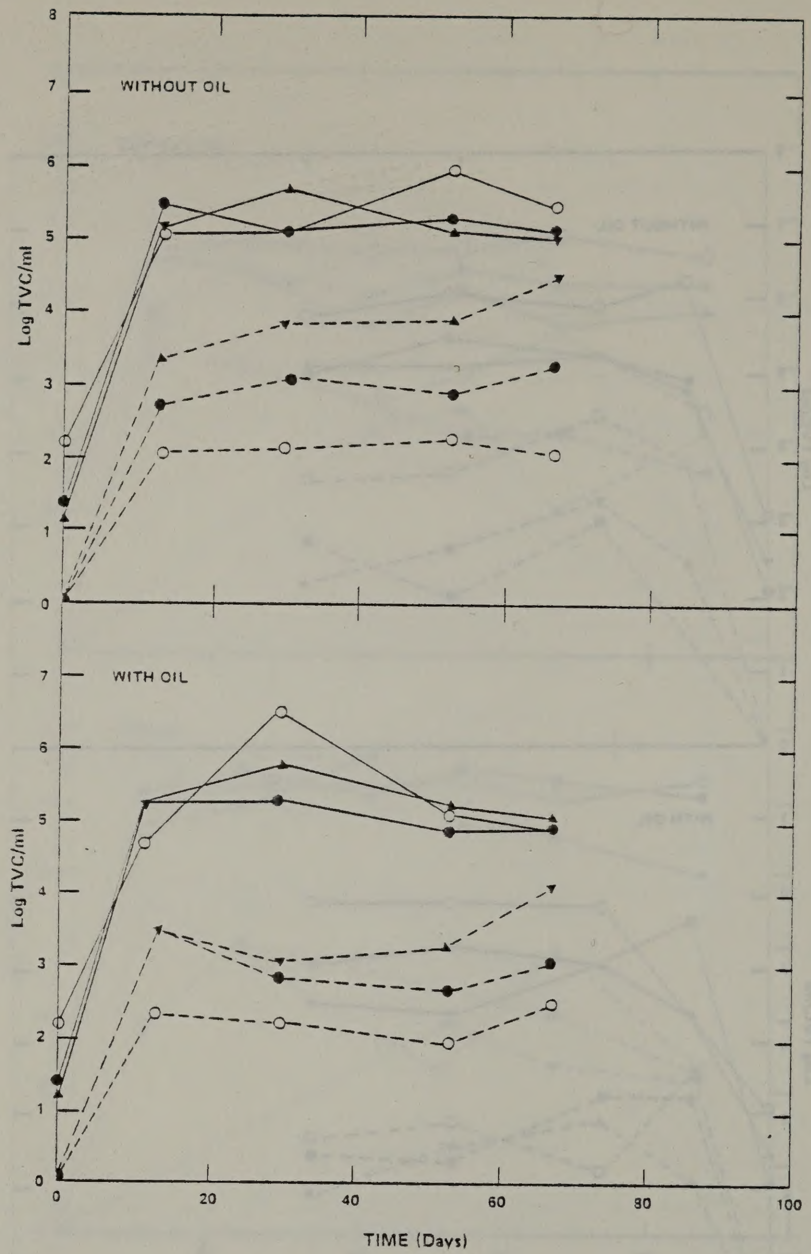


Figure 8.27 Microbial Populations (TVC-= total viable counts) in Water Samples Collected on the July Cruise (July 9). Samples were Incubated With or Without 0.5% SLCO. Solid Lines Indicate Total Marine Heterotrophs and Broken Lines Indicate Oil Degraders.
○ = Station 1; ● = Station 2; ▲ = Station 3

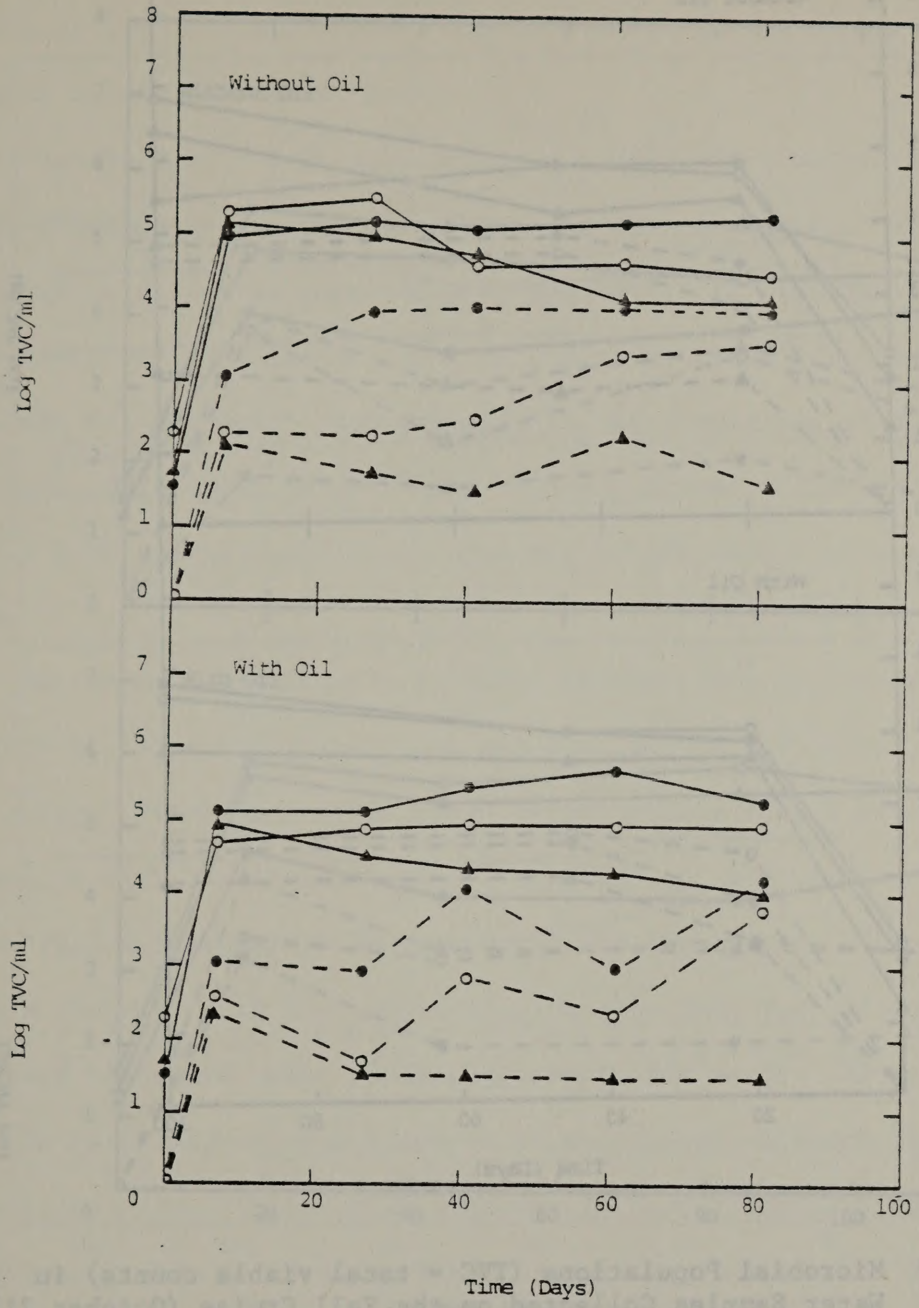


Figure 8.28 Microbial Populations (TVC = total viable counts) in Water Samples Collected on the August Cruise (August 6). Samples were Incubated With or Without 0.1% SLCO. Solid Lines Indicate Total Marine Heterotrophs and Broken Lines Indicate Oil Degraders.
○ = Station 1; ● = Station 2; ▲ = Station 3

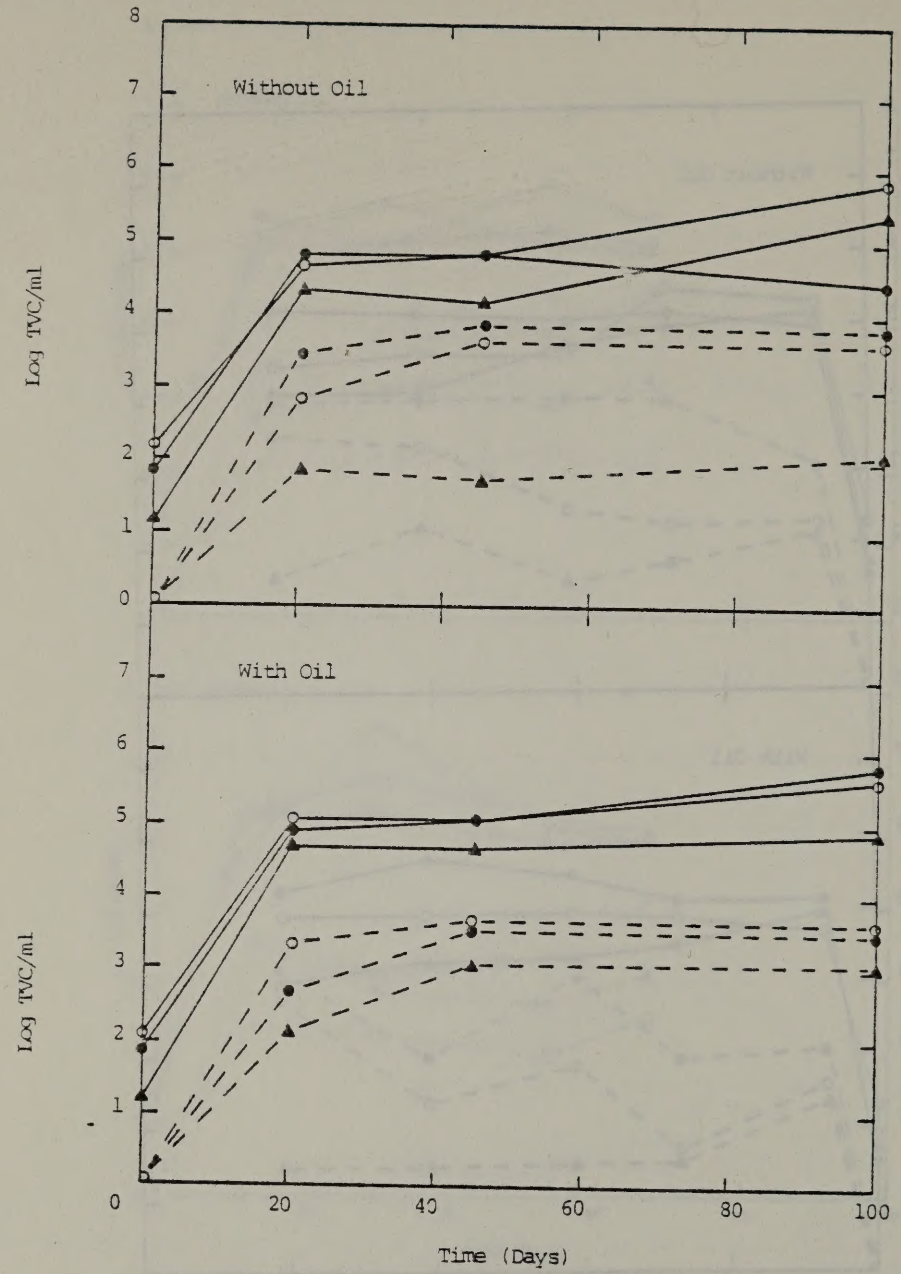


Figure 8.29 Microbial Populations (TVC = total viable counts) in Water Samples Collected on the Fall Cruise (October 21). Samples were Incubated With or Without 0.1% SLCO. Solid Lines Indicate Total Marine Heterotrophs and Broken Lines Indicate Oil Degraders.
○ = Station 1; ● = Station 2; ▲ = Station 3

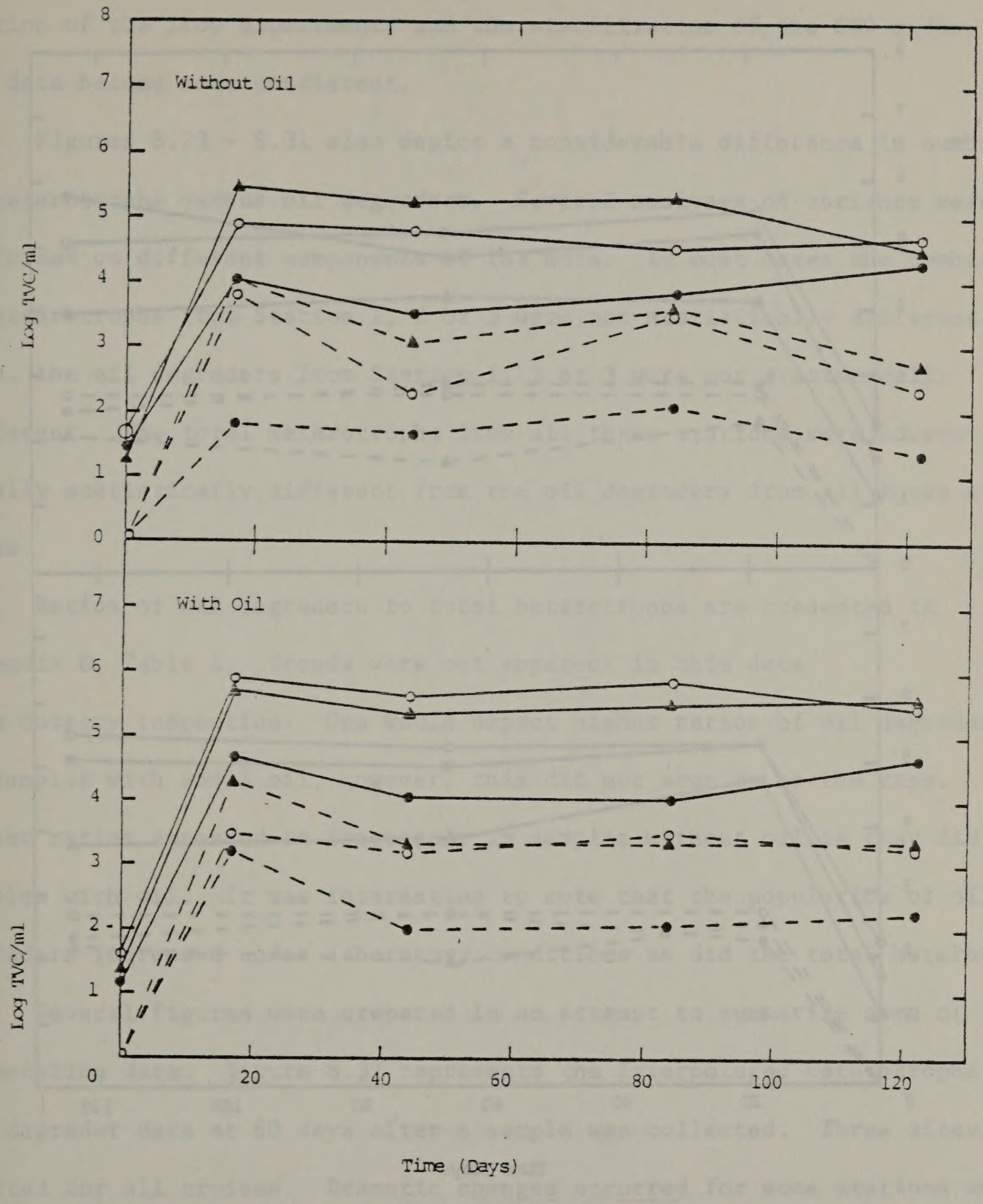


Figure 8.30 Microbial Populations (TVC = total viable counts) in Water Samples Collected on the November Cruise (November 20). Samples were Incubated With or Without 0.1% SLCO. Solid Lines Indicate Total Marine Heterotrophs and Broken Lines Indicate Oil Degraders.
○ = Station ; ● = Station 2; ▲= Station 3

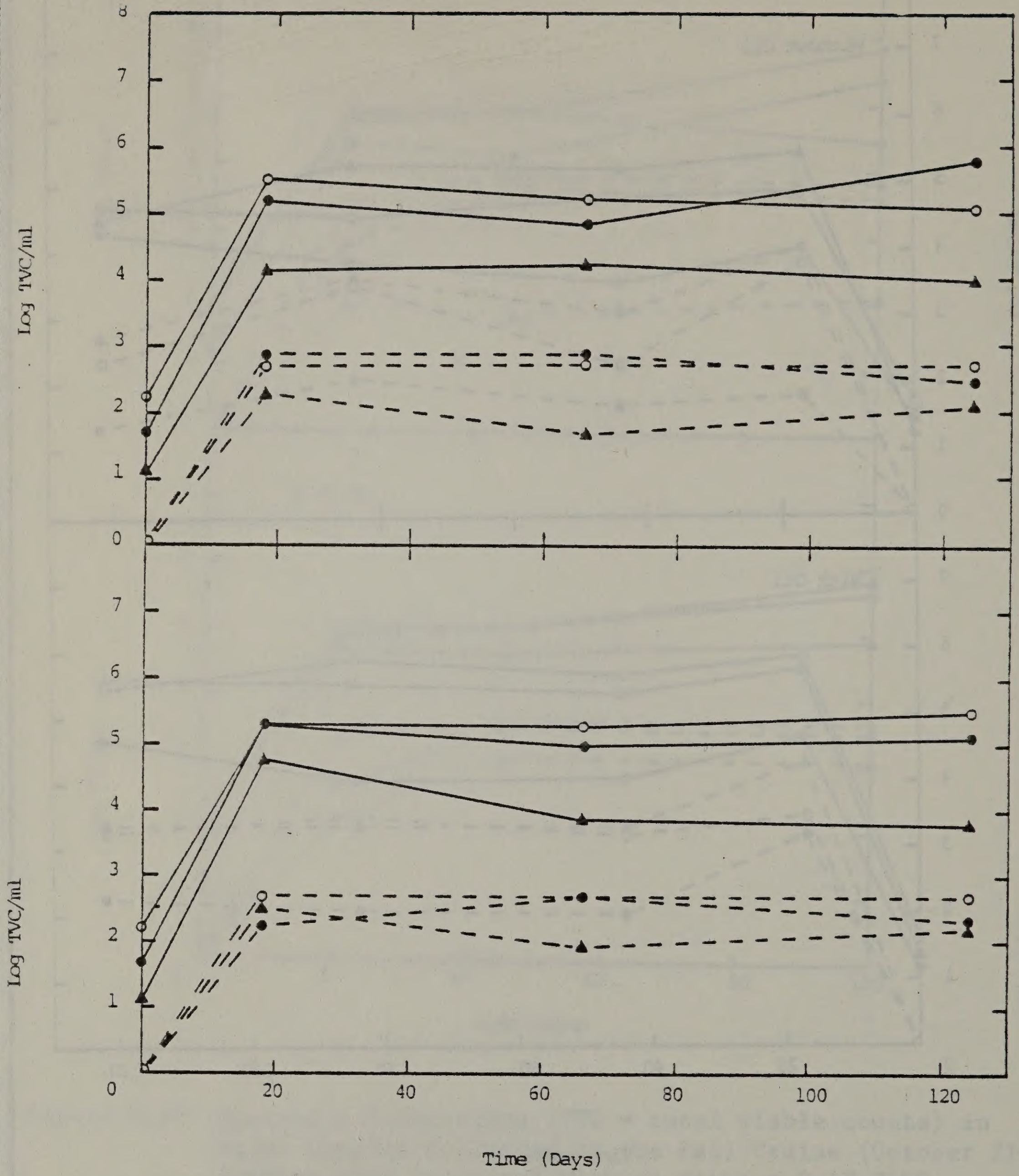


Figure 8.31 Microbial Populations (TVC = total viable counts) in Water Samples Collected on the December Cruise (December 16). Samples were Incubated With or Without 0.1% SLCO. Solid Lines Indicate Total Marine Heterotrophs and Broken Lines Indicate Oil Degraders.
○ = Station 1; ● = Station 2; ▲ = Station 3

cell population. Data for the first few cruises were quite erratic in some cases (especially the April cruise, Figure 8.25), but with the completion of the iron experiments and the stabilization of the SGO media, the data became more consistent.

Figures 8.23 - 8.31 also depict a considerable difference in number of heterotrophs versus oil degraders. Several analyses of variance were performed on different components of the data. In most cases the number of heterotrophs from Station 1, 2 or 3 were not statistically different. Also, the oil degraders from Station 1, 2 or 3 were nor statistically different. The total heterotrophs from all three stations were however usually statistically different from the oil degraders from all three stations.

Ratios of oil degraders to total heterotrophs are presented in Appendix G, Table 1. Trends were not apparent in this data upon cursory inspection. One would expect higher ratios of oil degraders in samples with added oil, however, this did not seem to be the case. Higher ratios appeared as frequently in samples without oil as they did in samples with oil. It was interesting to note that the population of oil degraders increased under laboratory conditions as did the total heterotrophs.

Several figures were prepared in an attempt to summarize some of the enumeration data. Figure 8.32 represents the interpolated heterotrophs and oil degrader data at 60 days after a sample was collected. Three sites are plotted for all cruises. Dramatic changes occurred for some stations on one or more cruises, but for the most part, seasonal trends, if present, were minor. Spatial trends were also minor. Degrees of significance will have to wait for the next phase of the project. Figure 8.33 depicts the same picture. Figures 8.34, 8.35 and 8.36 represent a comparison of oil treatment and control populations for all cruises. Points represent 60

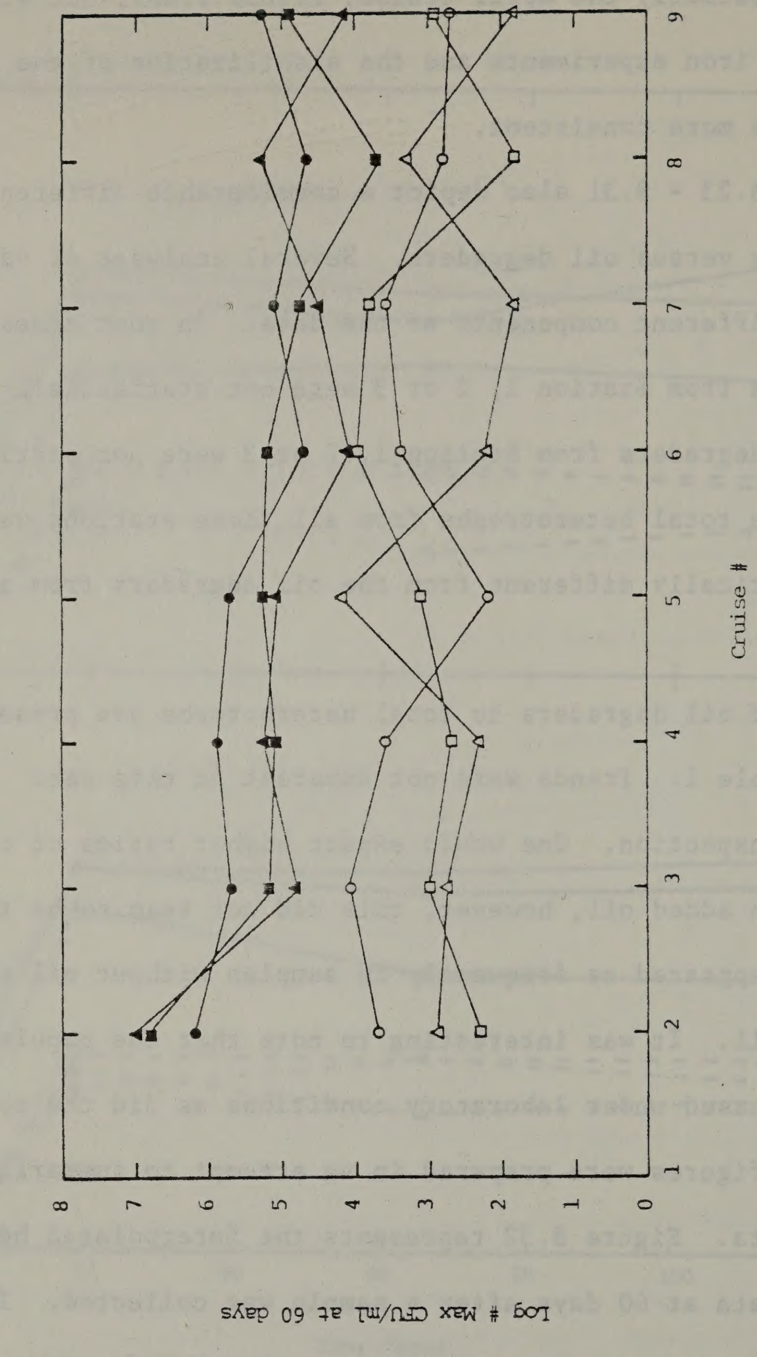


Figure 8.32 Log Number of CFU/ml 60 Days After Sampling. Samples Were Collected on Transect II: ○ = Site 1; □ = Site 2; △ = Site 3, and Incubated Without Oil. Solid Symbols are Total Heterotrophs; Open Symbols are Oil Degraders.

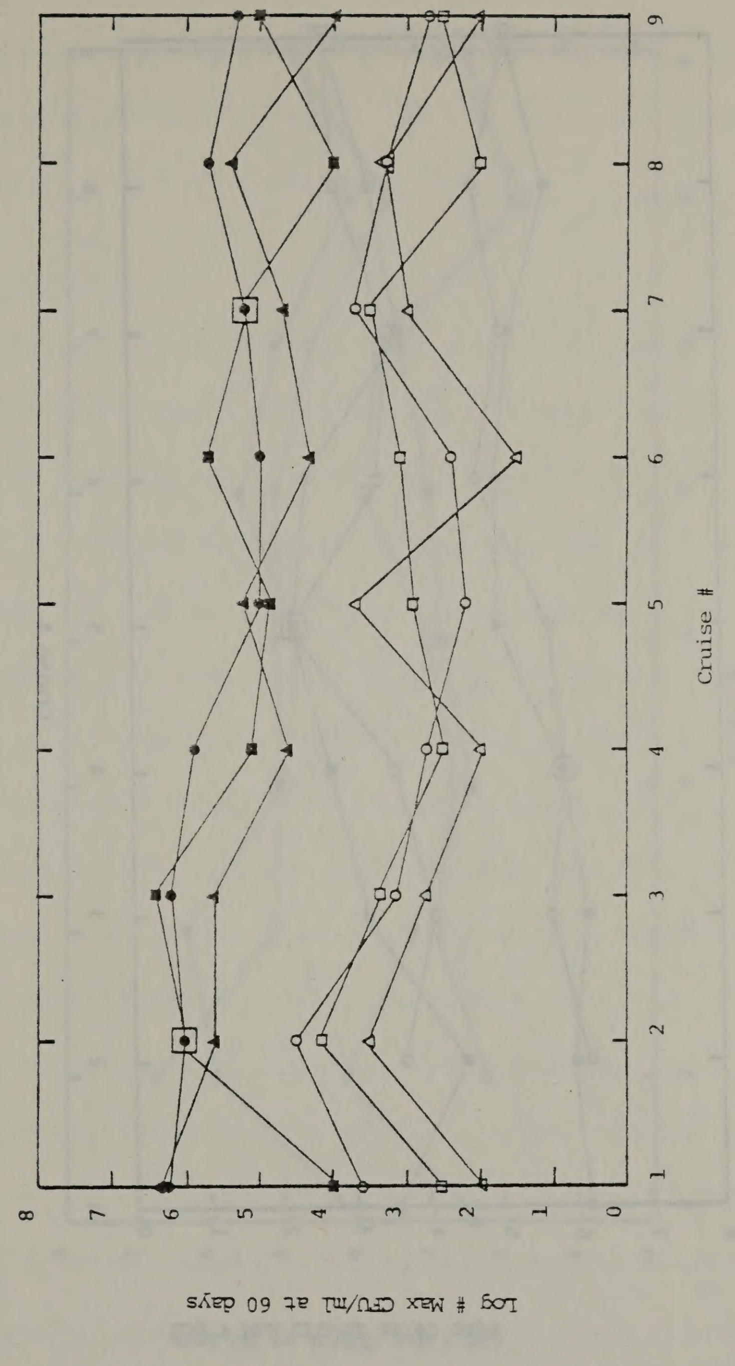


Figure 8.33 Log Number of CFU/ml 60 Days After Sampling. Samples Were Collected on Transect II: ○ = Site 1; □= Site 2; △ = Site 3, and Incubated With 0.5% SLCO (Winter, March, April, Spring and July Cruises) or 0.1% SLCO (August, Fall, November and December Cruises).

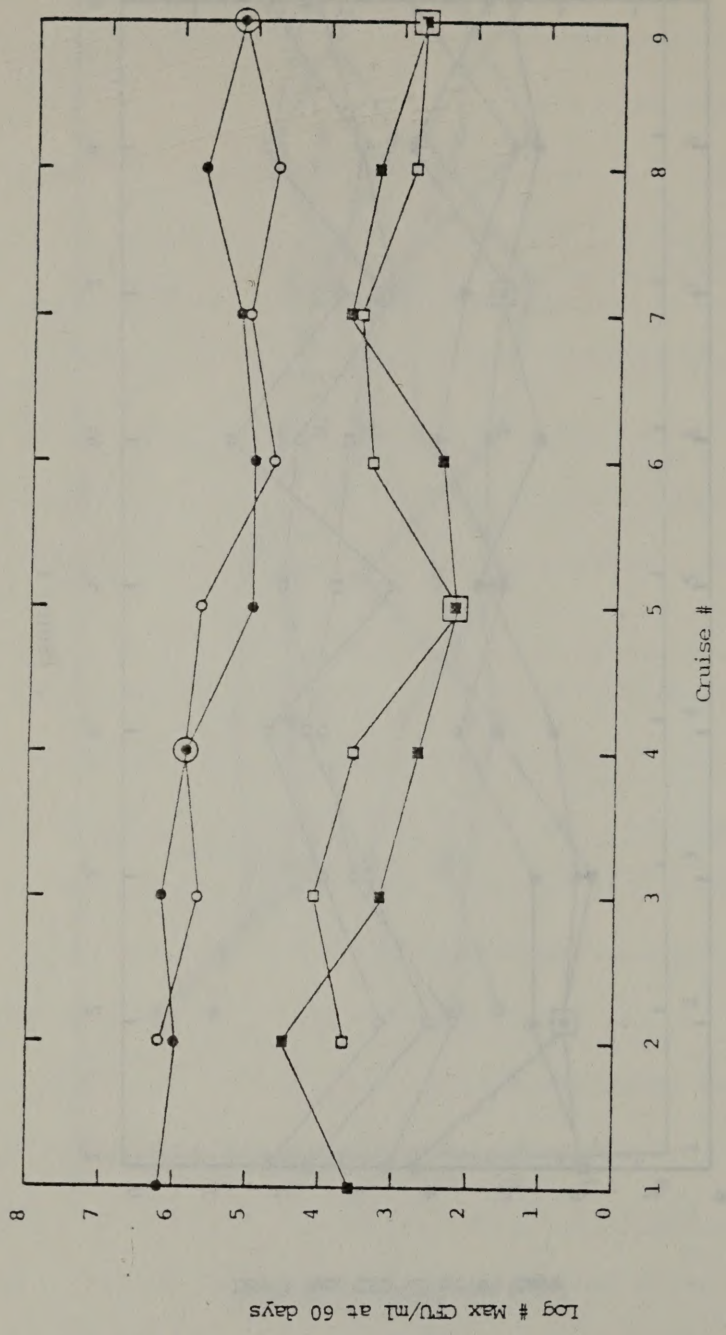


Figure 8.34 Log Number of CFU/ml 60 Days After Sampling. Samples Collected at Site 1, Transect II. Symbols are as Follows: ● = Total Heterotrophs, Incubated with SLCO; ○ = Total Heterotrophs Incubated Without SLCO; ■ = Oil Degraders Incubated With SLCO; □ = Oil Degraders Incubated Without SLCO.

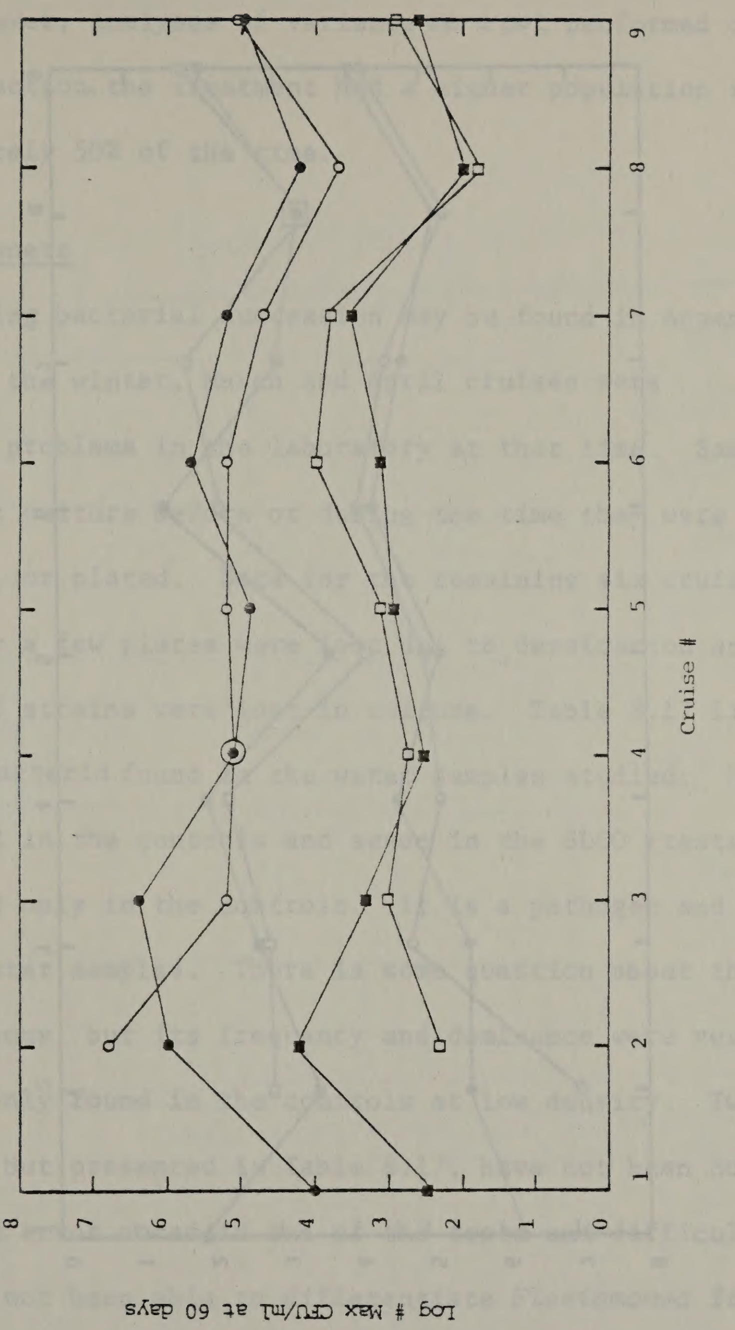


Figure 8.35 Log Number of CFU/ml 60 Days After Sampling. Samples Collected at Site 2, Transect II. Symbols are as Follows: ● = Total Heterotrophs, Incubated With SLCO; ○ = Total Heterotrophs Incubated Without SLCO; ■ = Oil Degraders Incubated with SLCO; □ = Oil Degraders Incubated Without SLCO.

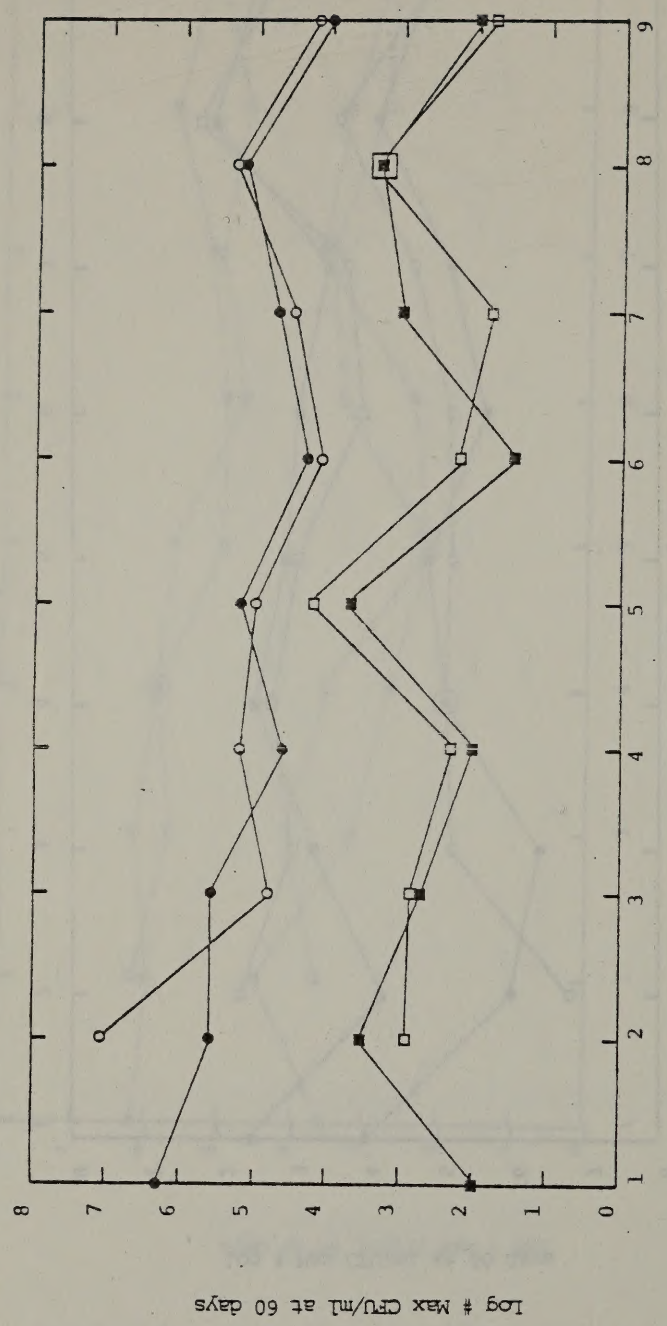


Figure 8.36 Log Number of CFU/ml 60 Days After Sampling. Samples Collected at Site 3, Transect II. Symbols are as Follows: ● = Total Heterotrophs, Incubated With SLCO; ○ = Total Heterotrophs Incubated Without SLCO; ■ = Oil Degraders Incubated With SLCO; □ = Oil Degraders Incubated Without SLCO.

days bacterial populations. The treatments and controls for both the total heterotrophs and oil degraders for sites 1, 2 and 3 were essentially the same, however, analyses of variance were not performed on all of the data. By inspection, the treatment had a higher population than the controls approximately 50% of the time.

Succession Genera

Data concerning bacterial succession may be found in Appendix G, Table 2. Data for the winter, March and April cruises were very meager due to problems in the laboratory at that time. Samples dried out or were lost in culture before or during the time they were worked up, enumerated, picked, or plated. Data for the remaining six cruises were more complete; only a few plates were lost due to dessication and a much lower proportion of strains were lost in culture. Table 8.17 lists the various genera of bacteria found in the water samples studied. Nine genera have been found in the controls and seven in the SLCO treated samples. *Moraxella* was found only in the controls. It is a pathogen and was not expected in salt water samples. There is some question about the identification of this genus, but its frequency and dominance were very low. *Bacillus* was also only found in the controls at low density. Two genera not included above but presented in Table 8.17, have not been confirmed as yet. Because of an error noted in one of the tests and difficulties with this test, we have not been able to differentiate *Plesiomonsa* from *Vibrio*, *Alcaligenes* and *Flavobacterium* and from several strains of the genus *Pseudomonas* (Table 8.18). The major genus found in all cases was *Pseudomonas*. A trend noted in some cases was the reduction in species richness that occurred in time as a sample was incubated in the laboratory. When colony types were used, many samples showed this trend (Figure 8.37 and 8.38). When the genera were plotted in the same manner and the unidentified strains

TABLE 8.17

GENERA OF BACTERIA FOUND IN THE CONTROL SAMPLES AND THOSE TREATED WITH SLCO

Genus	Control	SLCO
Pseudomonas	X	X
Flavobacterium	X	X
Acinotobacter	X	X
Coryneform	X	X
Actinomycetes	X	X
Moraxella*	X	0
Vibrio	X	X
Bacillus	X	0
Micrococcus	X	X
Plesiomonas	+	+
Alcaligenes	+	+

+ Identification not confirmed at this time.
* Pathogen, not expected in salt water samples.

TABLE 8.18

CRITERIA FOR THE IDENTIFICATION OF *Pseudomonas*, *Alcaligenes* AND *Flavobacterium* STRAINS.

Strain	Pigment	Oxidase	Of Glucose	Urease	Citrate	Flagella
Pseudomonas #1	Absent	–	i	∓	∓	Polar
Pseudomonas #2	Yellow	–	i	∓	∓	Polar
Pseudomonas #3	Absent	+	o	∓	∓	Polar
Pseudomonas #4	Absent	+	i	+	+	Polar
Pseudomonas #5	Absent	+	i	+	–	Polar
Unknown Ps #5*	Various	+	i	+	–	Polar or Peritrichous
Unknown Ps #6**	Absent	+	i	–	–	Polar or Peritrichous
Unknown Ps #7*	Various	+	i	–	–	Polar or Peritrichous
Unknown Ps #8**	Absent	+	i	–	+	Polar or Peritrichous
Flavobacterium #1	Yellow	+	i	∓	∓	Non-motile
Flavobacterium #2	Orange	+	i	∓	∓	Non-motile
Flavobacterium #3	Pink--Red	+	i	∓	∓	Non-motile

* Non-Pigmented Pseudomonads are not separated from genus Alcaligenes.
** Motile Flavobacterium are undifferentiated from pigmented Pseudomonas species. Flagella staining is in progress.
o = oxidative
i = inactive
+ = positive
– = negative

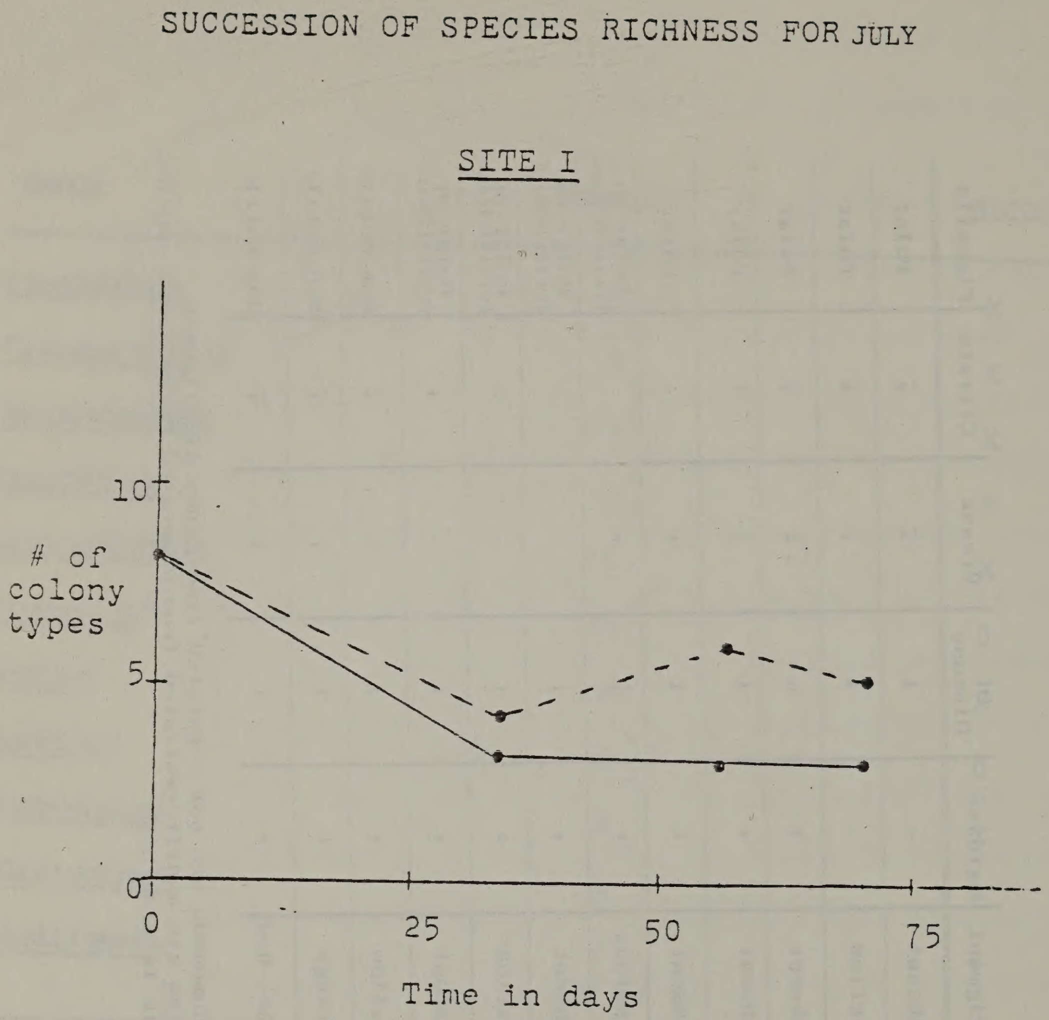


Figure 8.37 The Number of Species is Defined by the Colonial Morphology on Difco 2216 Agar for Marine Heterotrophs. Samples were Plated at Two Week Intervals. The Solid Line (———) Represents the Data Obtained from Flasks Incubated with 0.5% South Louisiana Crude Oil. The Dashed Line (- - - -) Represents the Data Obtained From the Control Flasks.

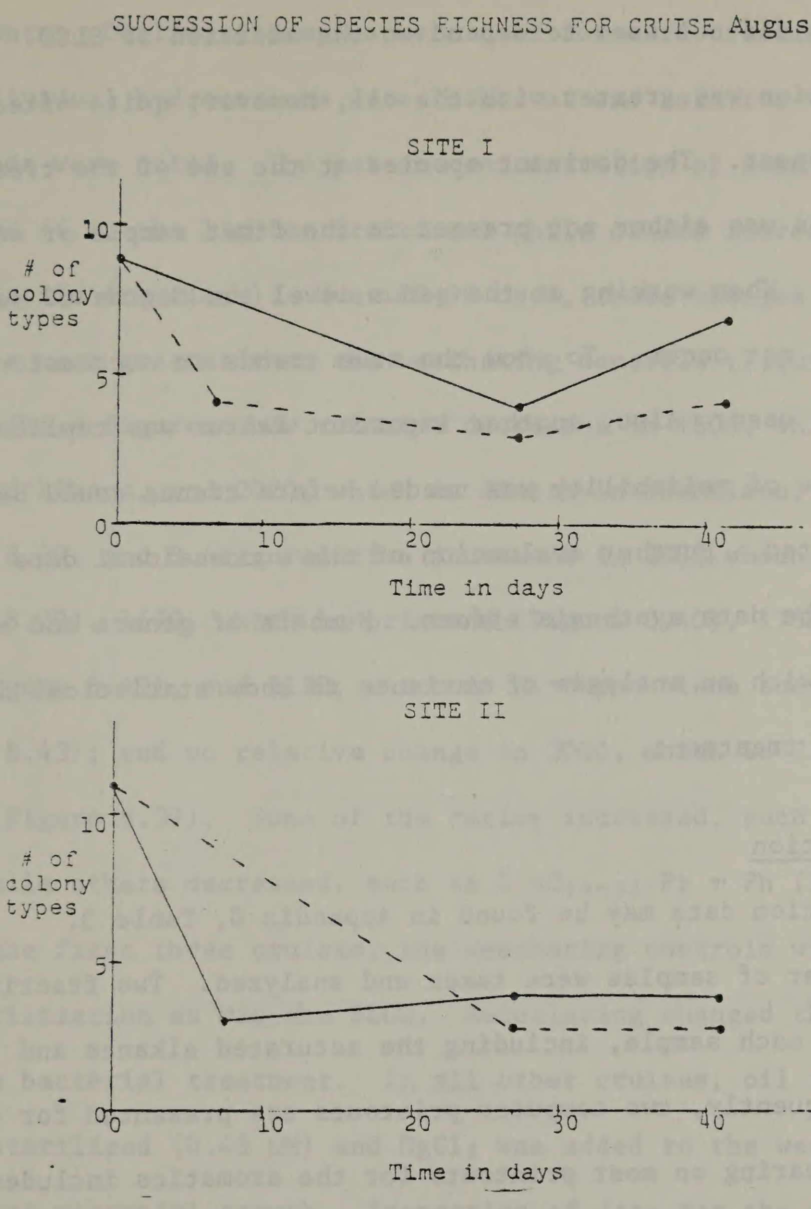


Figure 8.38 The Number of Species is Defined by the Colonial Morphology on Difco 2216 Agar for Marine Heterotrophs. Samples were plated at Two Week Intervals. The Solid Line (——) Represents the Data Obtained from Flasks Incubated with 0.5% SLCO. The Dashed Line (----) Represents the Data Obtained from the Control Flasks.

were included, some samples showed the same trends.

Differences did not seem to depend on the addition of SLCO. In some cases simplification was greater with the oil, however; quite often these trends did not appear. The dominant species at the end of the treatment time in some cases was either not present in the first sample or was of minor importance. When working at the genus level the degree of sensitivity needed did not occur. To show the true trends in succession the species level was used. Also, another important factor was replication. A greater estimate of reliability was needed before trends could be confirmed and predicted. Further evaluation of the successional data will be completed in the data synthesis effort. Numbers of genera and strains will be compared with an analysis of variance to show statistical differences in time and treatment.

Oil Degradation

Oil degradation data may be found in Appendix G, Table 3. A very large number of samples were taken and analyzed. Two fractions were included for each sample, including the saturated alkanes and the aromatics. Consequently, two computer printouts are presented for each sample. Data appearing on most printouts for the aromatics includes name (a retention index number), percent composition and component weight in micrograms. Some printouts do not have weights, but those weights are available and will be evaluated during the data synthesis effort. Data appearing on most printouts for the saturated hydrocarbons include name (a retention index number), percent composition (1100-3200), percent of 1600-3200, component weight in micrograms, pristane/phytane (Pr/Ph), Pr/1700, Ph/1800, and the sum of the n-alkanes 1600-3200/Pr + Ph.

Data for the Winter, March and April cruises include zero time samples, maximum time weathering controls (sterile samples with oil added), intermediate time samples as well as samples taken at the end of the experiments. Duplicate samples were used for each treatment and control. When individual hydrocarbons were looked at and relative values were plotted, trends were noted. The percent concentration of some components decreased (*i.e.* the lighter fractions) while others increased (the heavier, less volatile fractions). Over 60-90 days, these changes were not large, and never were greater than the weathering controls (Figures 8.39 through 8.43). These figures show relative decreases in 1600, which is C16, a saturated alkane, and 2020, which is dimethylnaphthalene, an aromatic (Figure 8.39 and 8.41); relative increases in 2400, which is normal C24 (Figure 8.39), 1670, which is pristane (Figure 8.40), 1780, which is phytane (Figure 8.40), and 2130, which is C_3-naphthalene and methylbiphenyl (Figure 8.43); and no relative change in 3000, which is C30, a saturated alkane (Figure 8.39). Some of the ratios increased, such as Pr/17 and Ph/18 while others decreased, such as $\Sigma\ nC_{16-32}/Pr + Ph$ (Figure 8.41). During the first three cruises, the weathering controls were autoclaved for sterilization as was the SLCO. Autoclaving changed the samples more than the bacterial treatment. In all other cruises, oil samples were filter sterilized (0.45 μM) and $HgCl_2$ was added to the weathering controls to prevent microbial growth. Inspection of data for the spring through December cruises indicated considerable variation, not only in total weights of hydrocarbon samples, but also in the weights and percentages of individual components. Ratios varied considerably as well, but not as would be expected if the samples were being actively metabolized.

Analyses of variance were performed on a number of parameters for several cruises, including total hydrocarbons, pristane/phytane ratios, weight of C_{16} and percent of C_{16}. No statistical differences were found.

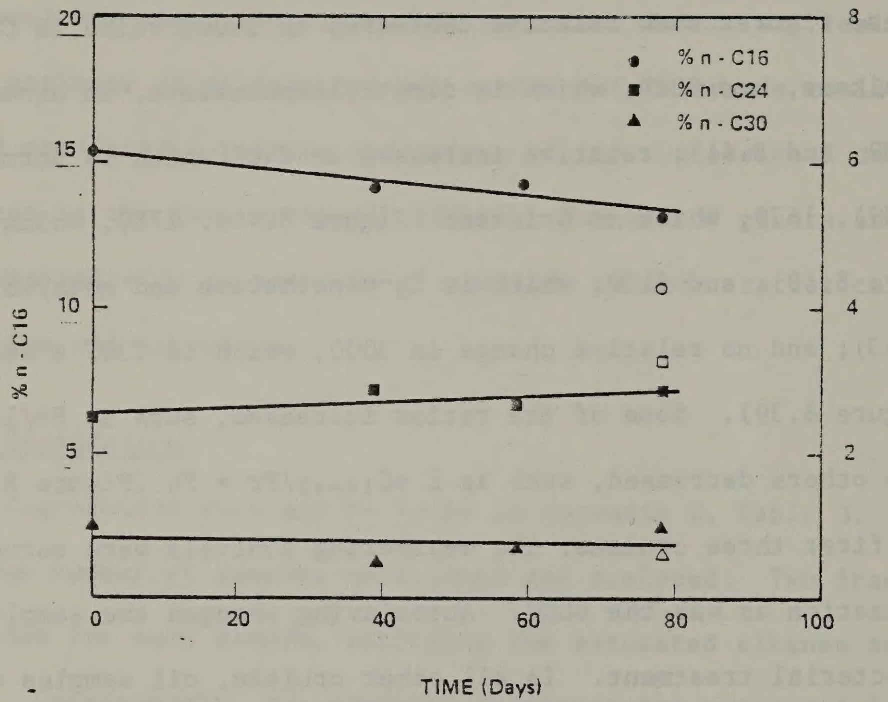


Figure 8.39 Average Percent of n-C16, n-C24, and n-C30 Found in All Incubated Degradation Samples for the Winter Cruise Plotted in Time. The Open Symbols are the Average Percent Weathering Control.

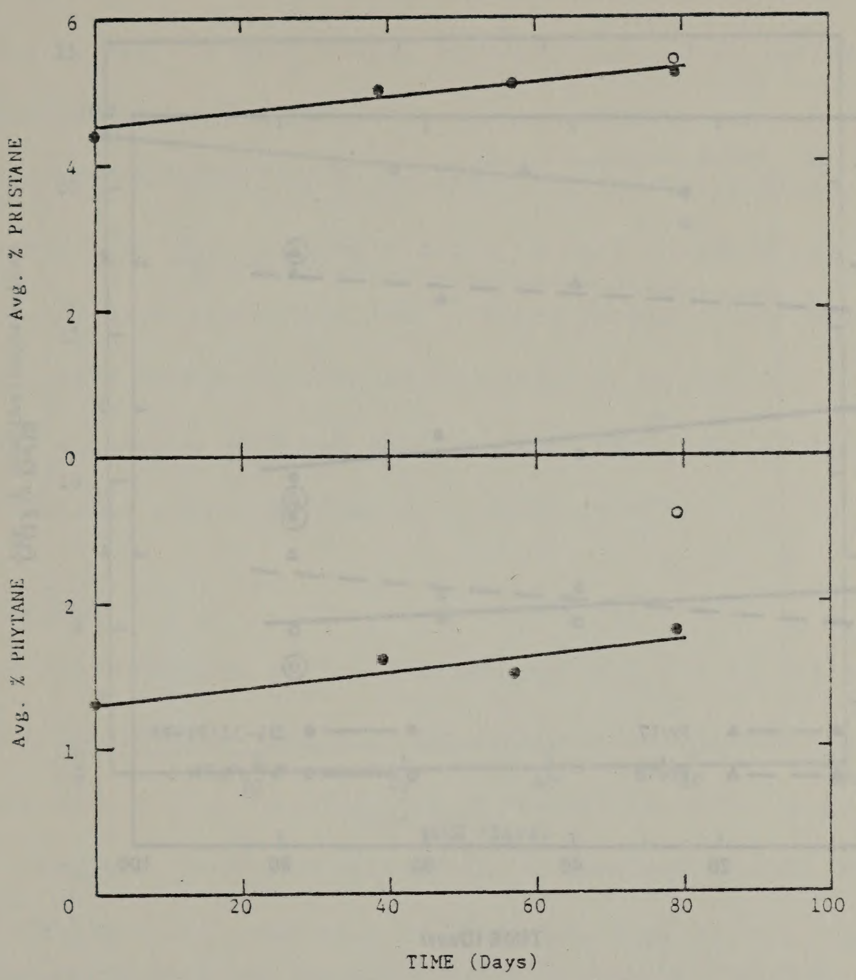


Figure 8.40 Average Percent Pristane and Phytane Found in all Incubated Degradation Samples for the Winter Cruise in Time. The Open Circle is the Average Percent Weathering Controls. Correlation Coefficients are 0.960 and 0.903, Respectively.

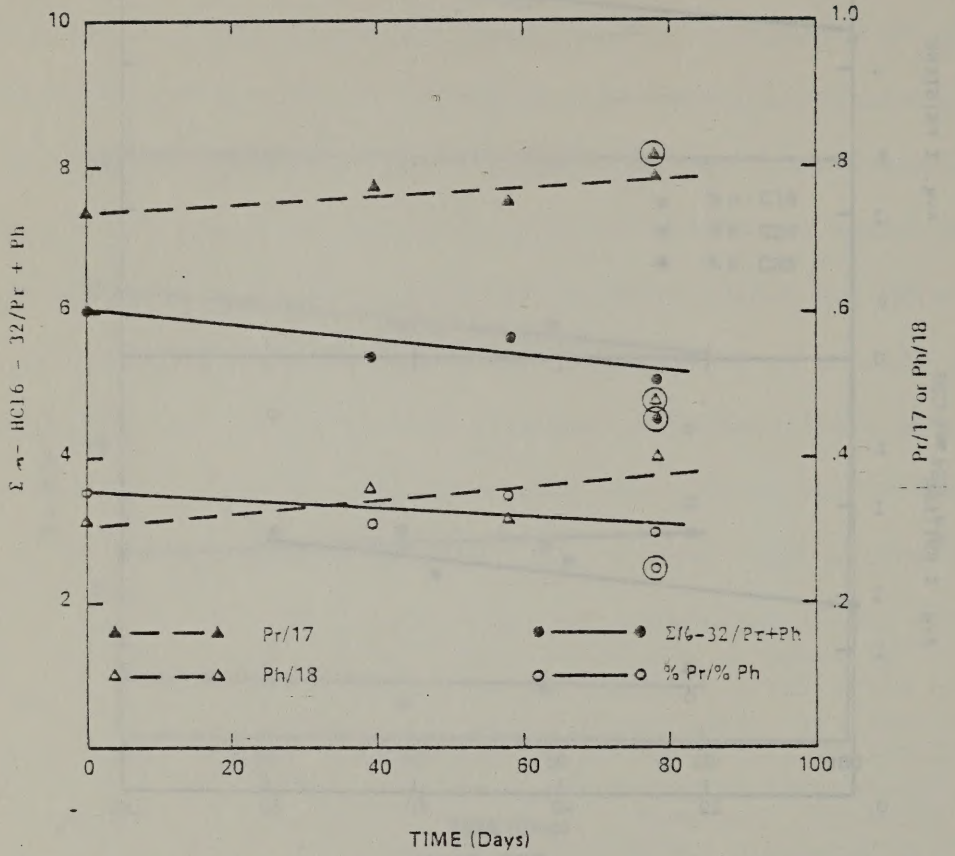


Figure 8.41 Average Pr/17, Ph/18, Pr/Ph and Σ n-HC 16-32/Pr + Ph Found in all Incubated Degradation Samples for the Winter Cruise Plotted in Time. The Circled Symbols are the Average Weathering Controls.

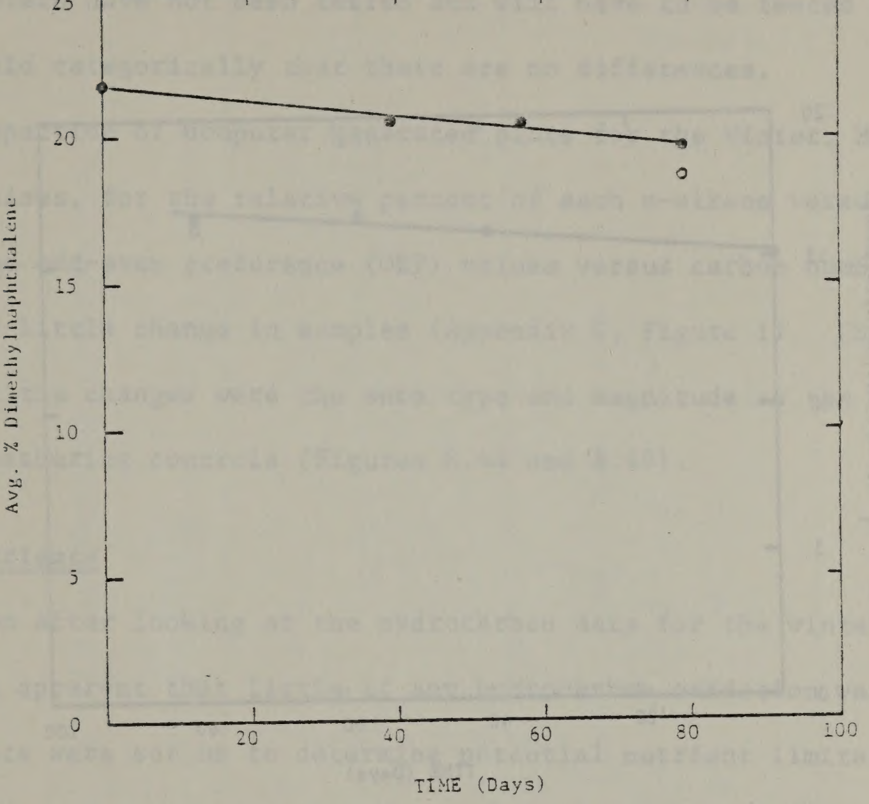


Figure 8.42 Average Percent of Dimethylnaphthalene Found in All Incubated Degradation Samples for the Winter Cruise Plotted In Time. The Open Symbols are the Average Weathering Controls. r = 0.984.

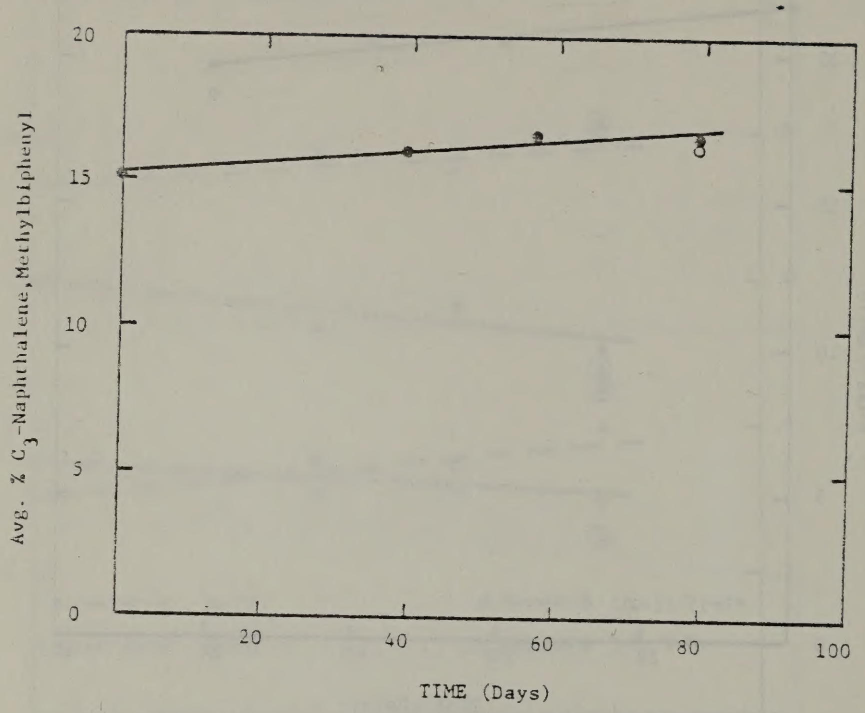


Figure 8.43 Average Percent of C_3-naphthalene and methylbiphenyl Found in All Incubated Degradation Samples for the Winter Cruise Plotted in Time. The Open Symbol is the Average Weathering Control. $r = 0.944$.

An example of the printout for the analysis of variance, the one tailed T-test comparisons and the average percent of C_{16} found in those samples may be found in Table 8.19. To date, no significant differences have appeared between the treatments and the controls, however; a great number of parameters have not been tested and will have to be tested before it can be said categorically that there are no differences.

Inspection of computer generated plots for the Winter, March and April cruises, for the relative percent of each n-alkane versus carbon number and odd-even preference (OEP) values versus carbon number also suggested little change in samples (Appendix G, Figure 1). Changes did occur but the changes were the same type and magnitude as the changes in the weathering controls (Figures 8.44 and 8.45).

Nutrients

Soon after looking at the hydrocarbon data for the winter cruise, it became apparent that little if any hydrocarbon oxidation was occurring. Experiments were set up to determine potential nutrient limitation of the seawater samples. Buchnell-Haas broth, a complete mineral media with 3.5% NaCl (Table 8.20), was used in these experiments, coupled with additions of 0.5% SLCO. Turbidimetric measurements were made for seawater samples from Station 1/I containing various ratios of Bushnell-Haas media. Figure 8.46 is a presentation of growth curves for various combinations with the BH media. When no oil was present, only straight turbidimetric changes occurred. When no BH media was present, the same thing occurred. When 5% and 50% BH media were present with SLCO, significant growth (optical change) occurred in 10-15 days. When the maximum optical density (OD) was plotted against the BH/seawater ratio (Figure 8.47), a drastic increase in the maximum OD occurred up to 10% BH ($\gamma = 0.929$).

TABLE 8.19

SAMPLE PRINTOUT OF THE ANALYSIS OF VARIANCE AND THE ONE TAILED T-TEST COMPARISONS CALCULATED FOR THE SPRING CRUISE SAMPLES, AND THE ESTIMATE OF MEAN PERCENT OF C16 FOUND IN THOSE SAMPLES

```
DEGRADE
****************************************************************************************************
****************************************************************************************************


ESTIMATES OF MEANS
------------------


                    T1          T2          T3          T4          T5          TOTAL
                       1           2           3           4           5           6

TIME        2       6.9500      7.1000      7.1500      7.1000      7.3500      7.1300


ONE WAY ANALYSIS OF VARIANCE FOR VARIABLE TIME
****************************************************************************************************


ANALYSIS OF VARIANCE

SOURCE OF VARIANCE            D.F.     SUM OF SQ.     MEAN SQ.     F-VALUE        TAIL AREA PROBABILITY
EQUALITY OF CELL MEANS          4        0.1660        0.0415       0.5845              0.6885
   ERROR                        5        0.3553        0.0710


T-TEST MATRIX FOR GROUP MEANS
-----------------------------


                    T1          T2          T3          T4          T5
                       1           2           3           4           5

T1          1       0.0
T2          2       0.4472      0.0
T3          3       0.9428      0.1491      0.0
T4          4       0.6000      0.0        -0.2000      0.0
T5          5       2.5298      0.8220      1.2649      1.2127      0.0


PROBABILITIES FOR THE T-VALUES ABOVE
------------------------------------


                    T1          T2          T3          T4          T5
                       1           2           3           4           5

T1          1       1.0000
T2          2       0.6985      1.0000
T3          3       0.4453      0.8952      1.0000
T4          4       0.6094      1.0000      0.8600      1.0000
T5          5
```

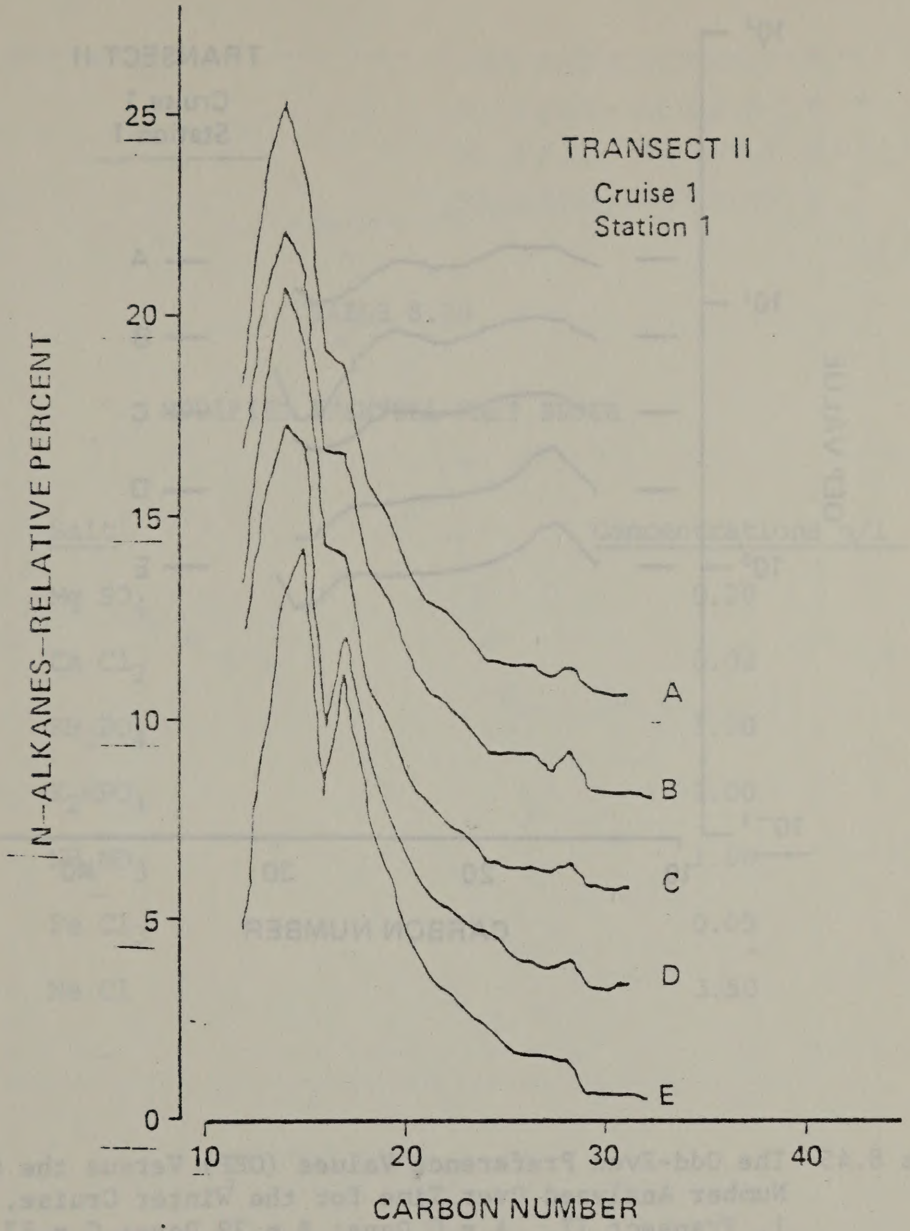


Figure 8.44 The Relative Percent of n-Alkanes Versus the Carbon Number Analyzed in Time for the Winter Cruise, Transect II, Station 1. A = 0 Day; B = 39 Days; C = 57 Days; D = 79 Days; E = Weathering Control at 79 Days.

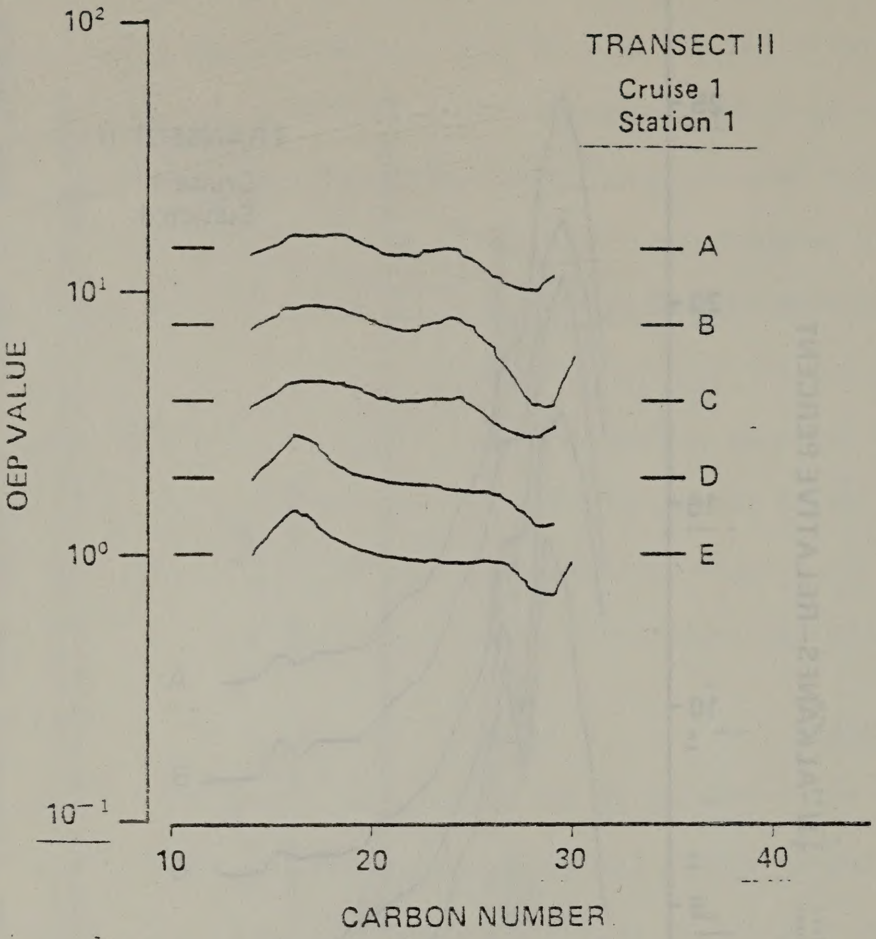


Figure 8.45 The Odd-Even Preference Values (OEP) Versus the Carbon Number Analyzed Over Time for the Winter Cruise, Station 1, Transect II. A = 0 Days; B = 39 Days; C = 57 Days; D = 79 Days; E = Weathering Control at 79 Days.

TABLE 8.20

MODIFIED BUSHNELL-HAAS BROTH

Salt	Concentrations g/l
$Mg\ SO_4$	0.20
$Ca\ Cl_2$	0.02
KH_2PO_4	1.00
K_2HPO_4	1.00
NH_4NO_3	1.00
$Fe\ Cl_3$	0.05
$Na\ Cl$	3.50

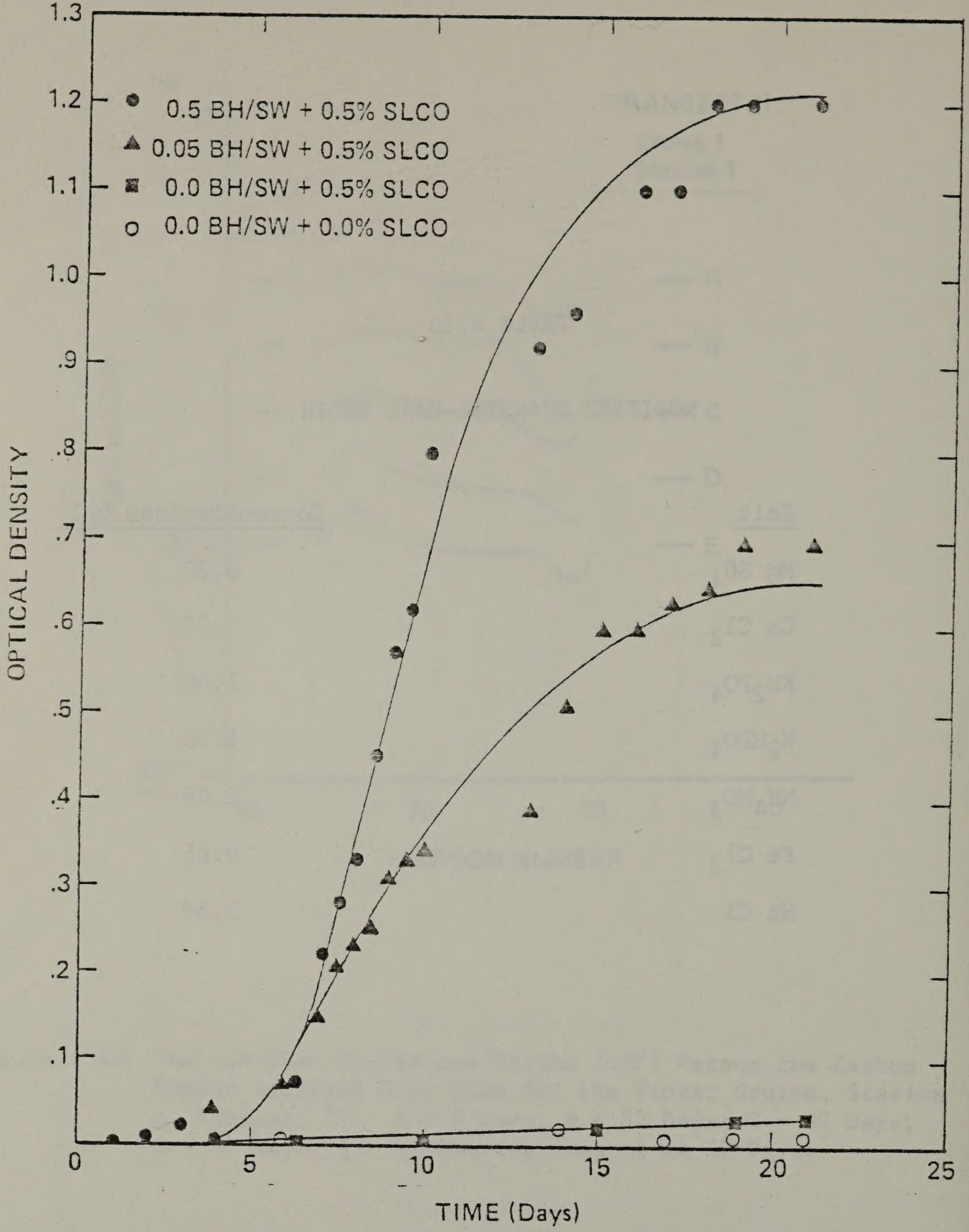


Figure 8.46 The Effect of SLCO and Various Ratios of Bushnell-Haas Media on the Growth Rate of Mixed Natural Bacterial Populations.

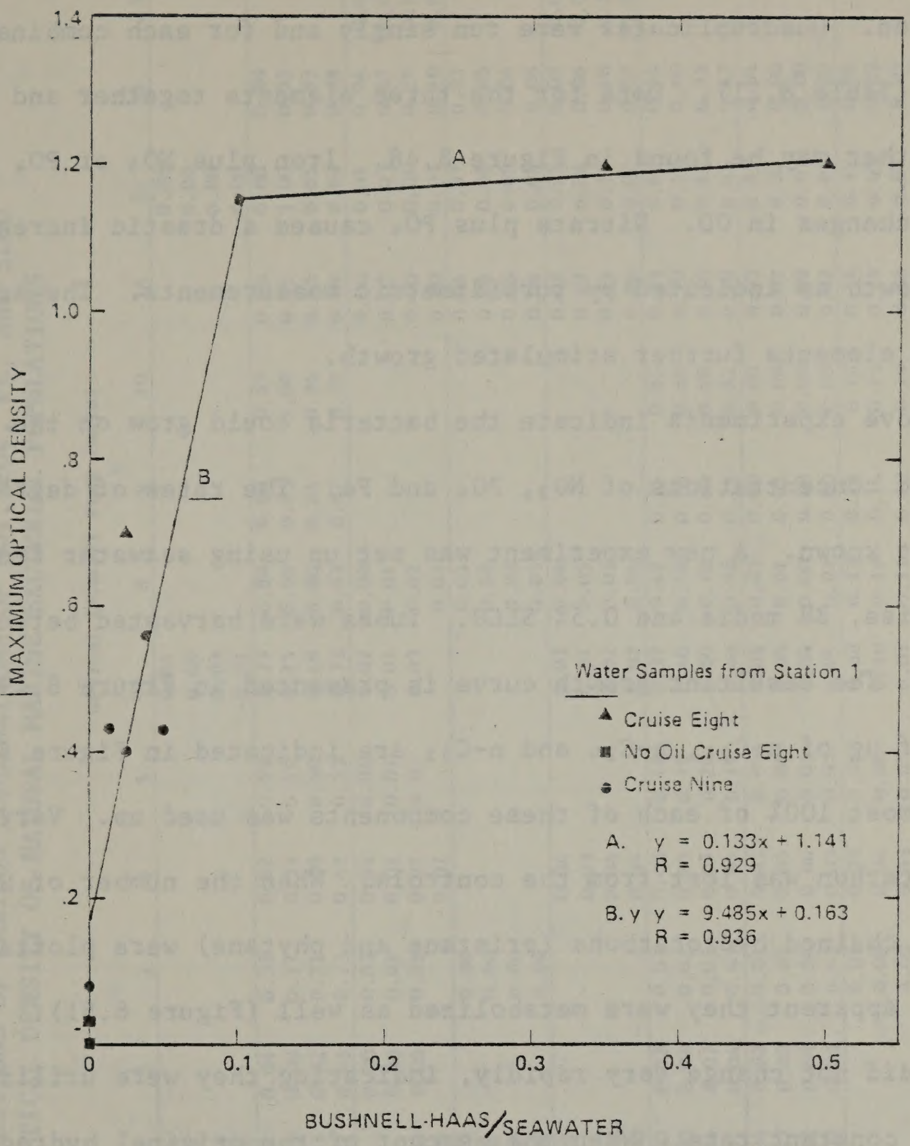


Figure 8.47 The Effect of Increasing Ratios of BH Media on the Maximum Optical Density of Natural Bacterial Populations.

The critical ratio of BH/seawater is 0.10. The specific mineral elements that were limiting were investigated as well. Nitrate, PO_4, and Fe were chosen. Quadruplicates were run singly and for each combination of elements (Table 8.21). Data for the three elements together and for any two together may be found in Figure 8.48. Iron plus NO_3 or PO_4 caused only slight changes in OD. Nitrate plus PO_4 caused a drastic increase in bacterial growth as indicated by turbidimetric measurements. The addition of all three elements further stimulated growth.

The above experiments indicate the bacteria could grow on the SLCO given certain concentrations of NO_3, PO_4 and Fe. The rates of degradation are still not known. A new experiment was set up using seawater from the November cruise, BH media and 0.5% SLCO. Tubes were harvested between 0 and 21 days. The resultant growth curve is presented in Figure 8.49. The number of µg of $n\text{-}C_{16}$, $n\text{-}C_{24}$ and $n\text{-}C_{32}$ are indicated in Figure 8.50. Note that almost 100% of each of these components was used up. Very little of the hydrocarbon was lost from the controls. When the number of µg from the branched chained hydrocarbons (pristane and phytane) were plotted in time, it was apparent they were metabolized as well (Figure 8.51). The Pr/Ph ratio did not change very rapidly, indicating they were utilized at a relatively constant rate. When the percent of the original hydrocarbon utilized was plotted for $n\text{-}C_{16}$, $n\text{-}C_{24}$, $n\text{-}C_{32}$ and pristane and phytane . (Figure 8.52), it was obvious that the three normal alkanes were used at the same rate, whereas the pristane and phytane were metabolized more slowly. Comparisons with weathering controls indicated only a small loss due to physical factors. When other alkanes were examined, such as $n\text{-}C_{17}$ and $n\text{-}C_{18}$, it was found they were rapidly metabolized as well (Figure 8.53). Ratios of Pr/17, Ph/18 and Σ n-alkanes/Pr + Ph also indicated a rapid

TABLE 8.21

EFFECT OF ADDITIONS OF VARIOUS COMBINATIONS OF NO_3, PO_4, FE, AND SLCO ON THE OPTICAL DENSITY OF NATURAL MARINE BACTERIAL POPULATIONS

NO_3^- g/ℓ	$PO_4^{\equiv}$ g/ℓ	Fe^{+++} g/ℓ	Percent SLCO*	OPTICAL DENSITY TIME (DAYS)													
				1	2	3	4	5	7	8	9	10	14	21	22	23	3
0.388	0.644	0.009	0.00	-0.01					-0.01					0.00			
				-0.01					0.00					0.00			
				-0.01					-0.03					0.00			
				-0.01					-0.01					0.00			
0.388	0.644	0.009	0.50	-0.03	0.04	0.32	0.52	0.59	0.73	0.80	0.80	0.85	0.80	0.80	0.80		
				-0.03	0.00	0.17	0.35	0.49	0.75	0.90	0.95	1.00	0.95	1.00	0.95		
				-0.03	0.02	0.21	0.40	0.50	0.68	0.80	0.85	0.85	0.85	0.85	0.85		
				-0.03	0.03	0.23	0.42	0.53	0.72	0.83	0.87	0.90	0.88	0.88	0.88		
0.388	–	–	0.50	–	0.00	0.02	0.01	0.04	0.07	0.00			0.00	0.01	0.04	0.05	
				–	0.00	0.01	0.01	0.01	0.01	0.01			0.02	0.03	0.07	0.07	
				0.00	0.00	0.01	0.01	0.02	0.03	0.02			0.00	0.06	0.24	0.14	
				0.00			0.01			0.01			0.00	0.03	0.10	0.08	
–	0.644	–	0.50	0.00		0.00				0.03			0.06	0.07	0.07		
				0.00		0.00				0.02			0.06	0.01	0.05		
				0.00		0.00				0.03			0.06	0.05	0.06		
				0.00		0.00				0.03			0.06	0.04	0.06		
–	–	0.009	0.50	0.01			0.04		0.03	0.04			0.07	0.05	0.05	0.06	
				–			0.03		0.01	0.05			0.07	0.06	0.06	0.06	
				0.00			0.04		0.02	0.03			0.05	0.10	0.10	0.11	
				0.00			0.04		0.02	0.04			0.06	0.07	0.07	0.08	
0.388	–	–	0.50	0.00	0.02	0.02	0.03	0.18	0.37	0.47	0.75	0.85	0.75	0.64	0.66		
				0.00	0.04	0.02	0.05	0.19	0.40	0.58	0.70	0.80	0.70	0.52	0.60		
				0.04	0.07	0.05	0.07	0.20	0.60	0.70	0.80	0.80	0.70	0.64	0.75		
				0.01	0.04	0.03	0.05	0.19	0.46	0.58	0.75	0.82	0.70	0.65	0.67		
0.388	–	0.009	0.50	0.04	0.02	0.04	0.05	0.05	0.04	0.03	0.00	0.00	0.03	0.02	0.01		
				0.02	0.02	0.05	0.05	0.04	0.05	0.01	0.01	0.00	0.02	0.02	0.00		
				0.02	0.04	0.06	0.06	0.05	0.04	0.00	0.00	0.00	0.00	0.03	0.00		
				0.02	0.03	0.05	0.05	0.05	0.04	0.02	0.00	0.00	0.02	0.02	0.00		
–	0.644	0.009	0.50			0.01	0.05	0.06	0.08	0.13	0.15	0.16	0.16	0.12	0.08		
						0.00	0.04	0.06	0.10	0.12	0.14	0.15	0.19	0.05	0.07		
						0.00	0.03	0.05	0.07	0.11	0.10	0.10	0.06	0.08	0.05		
						0.00	0.03	0.06	0.08	0.12	0.13	0.14	0.14	0.08	0.07		

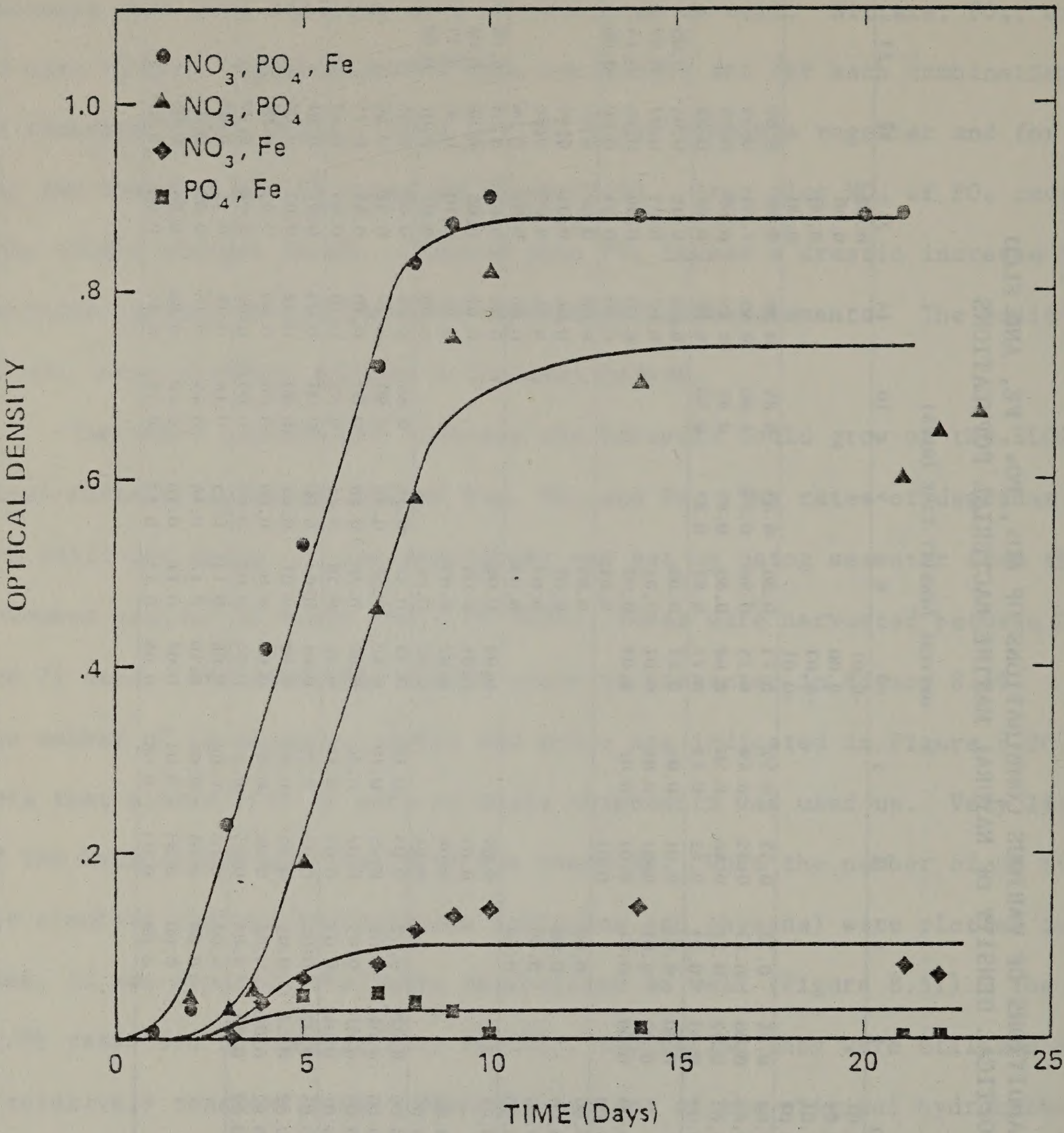


Figure 8.48 Growth of Marine Bacteria (Increase in Outside Diameter) in Time as Effected by Combinations of NO_3, PO_4, and Fe.

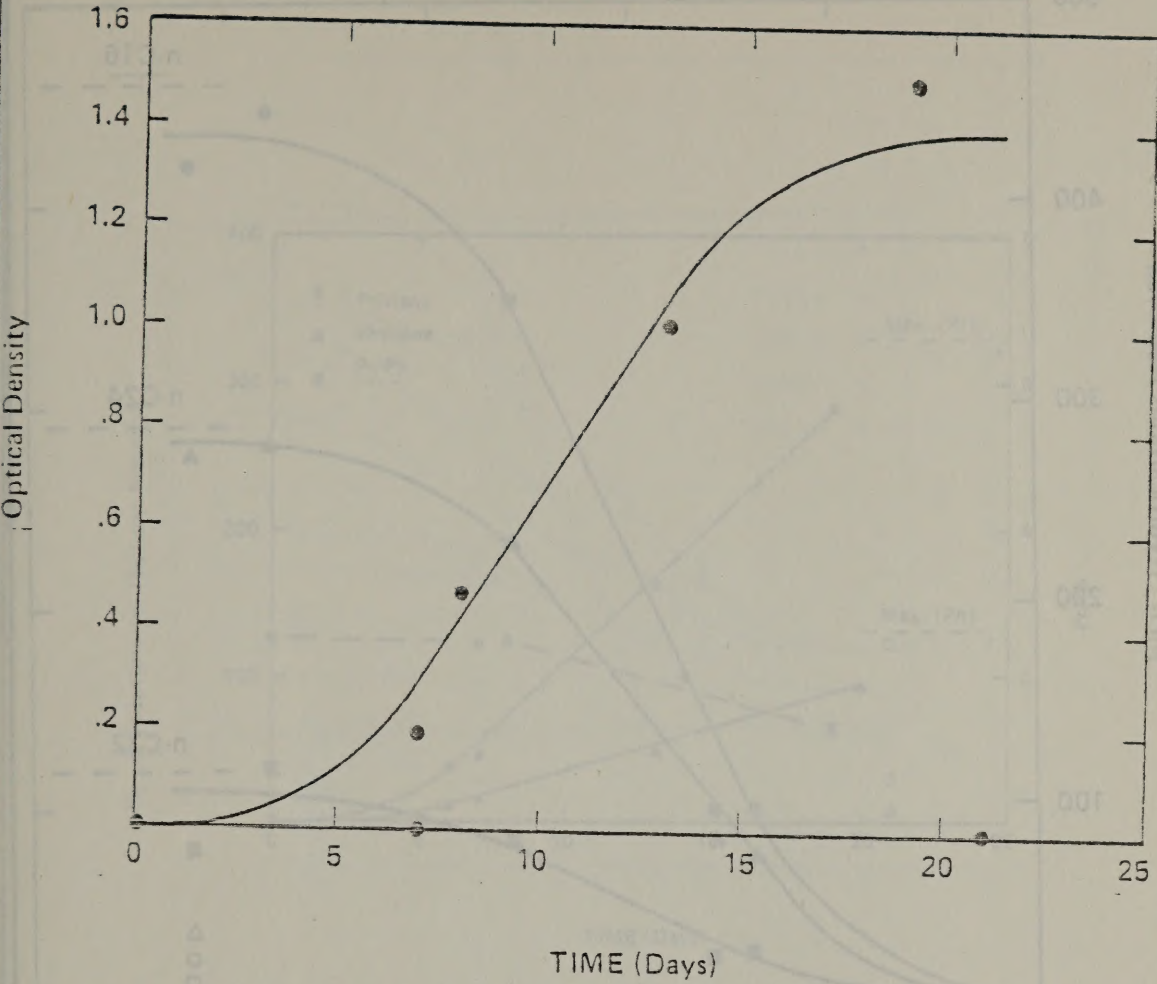


Figure 8.49 Charge in Outside Diameter of a Natural Marine Bacterial Population in Time. Bacteria Were Grown in BH Media.

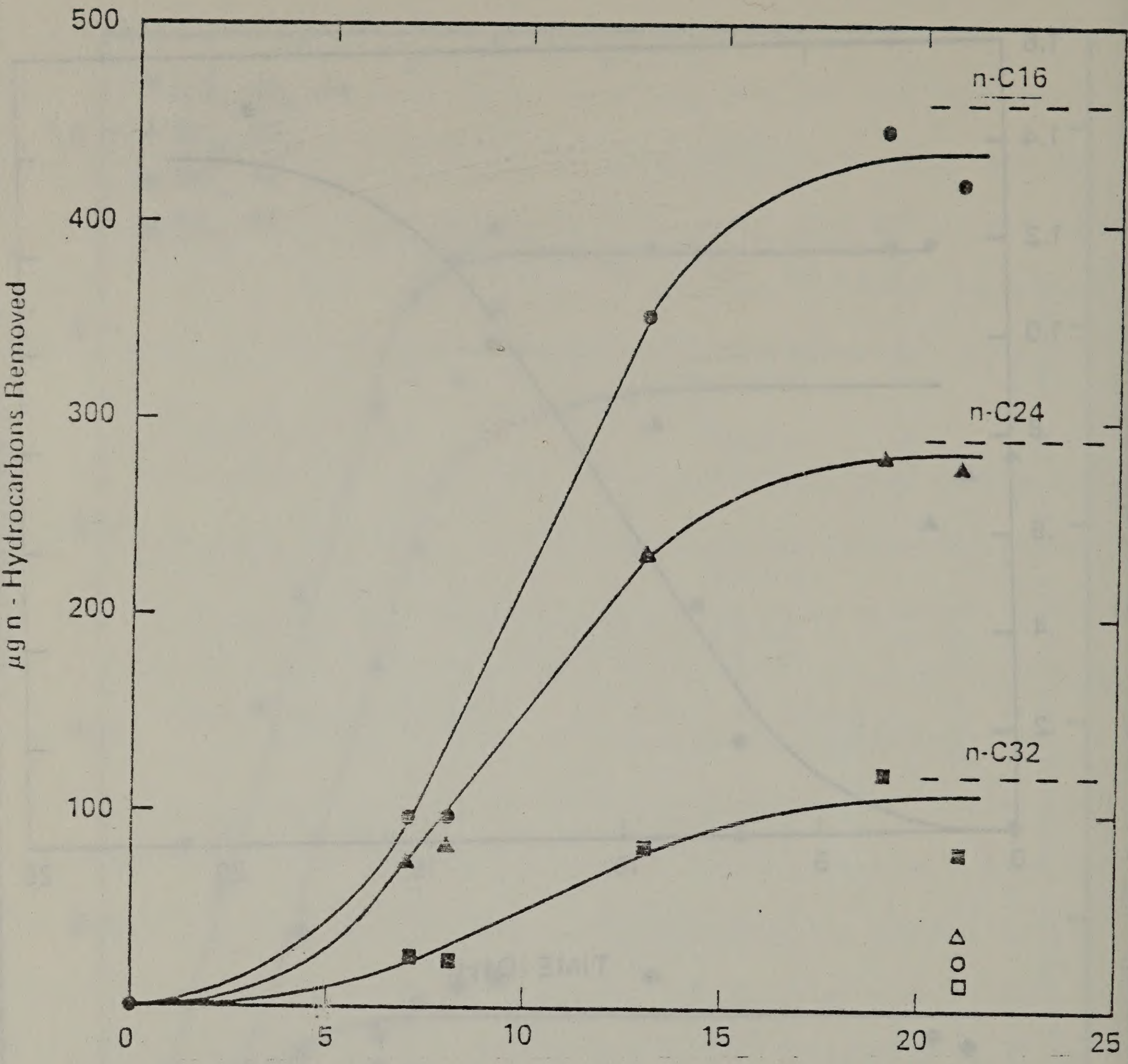


Figure 8.50 The Metabolism n-C16, n-C24, and n-C32 by Natural Populations of Marine Bacteria. Points in the Figure are x̄ of the Amount of the Above Alkanes Removed from a Treatment Tube in Time. Open Symbols are Controls. Dashed Lines Represent Zero Time Maximum Values.

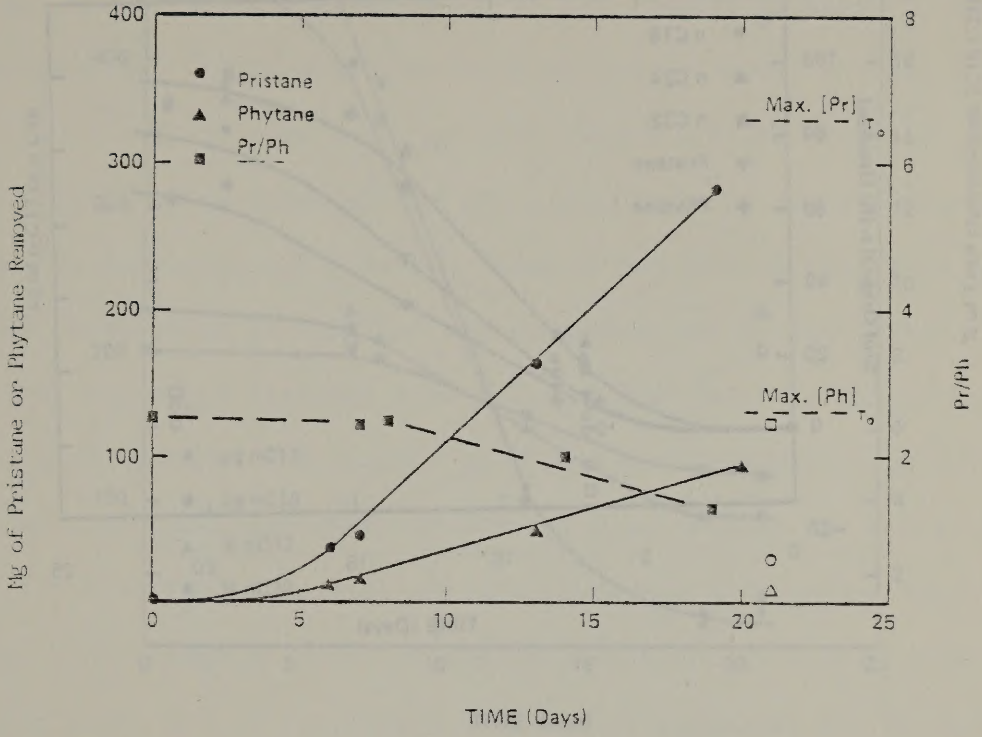


Figure 8.51 The Metabolism of Pristane and Phytane by Natural Populations of Marine Bacteria. Points are $\bar{x}$. Open Symbols are Controls and Light Dashed Lines are Maximum Values. The Negative Slope of the PR/PH Ratio Indicates a More Rapid Rate of Metabolism for the Pristane.

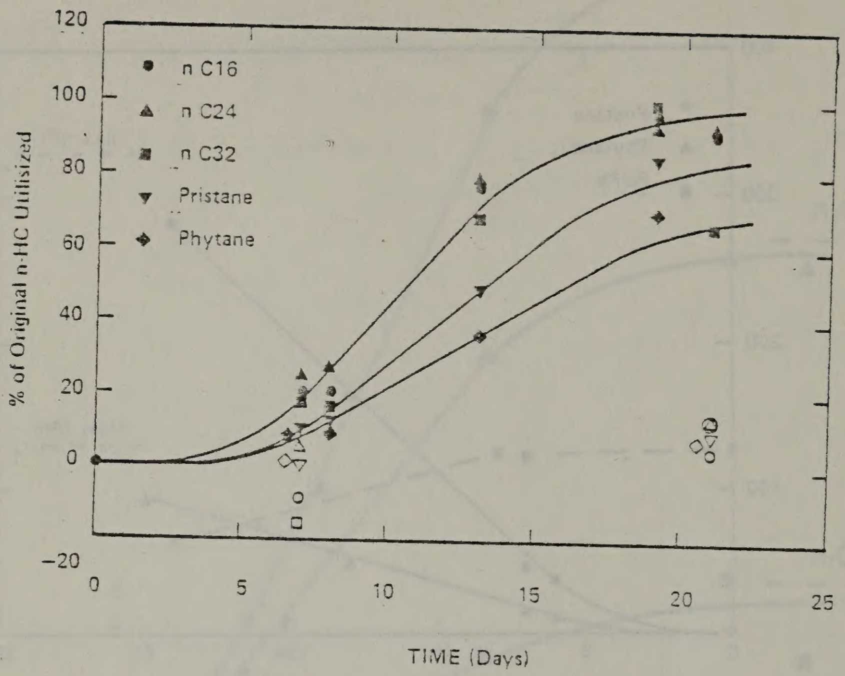


Figure 8.52 Utilization of Normal and Branched Alkanes by Natural Marine Bacterial Populations Presented as a Percent of the Original Material. Open Symbols are Controls.

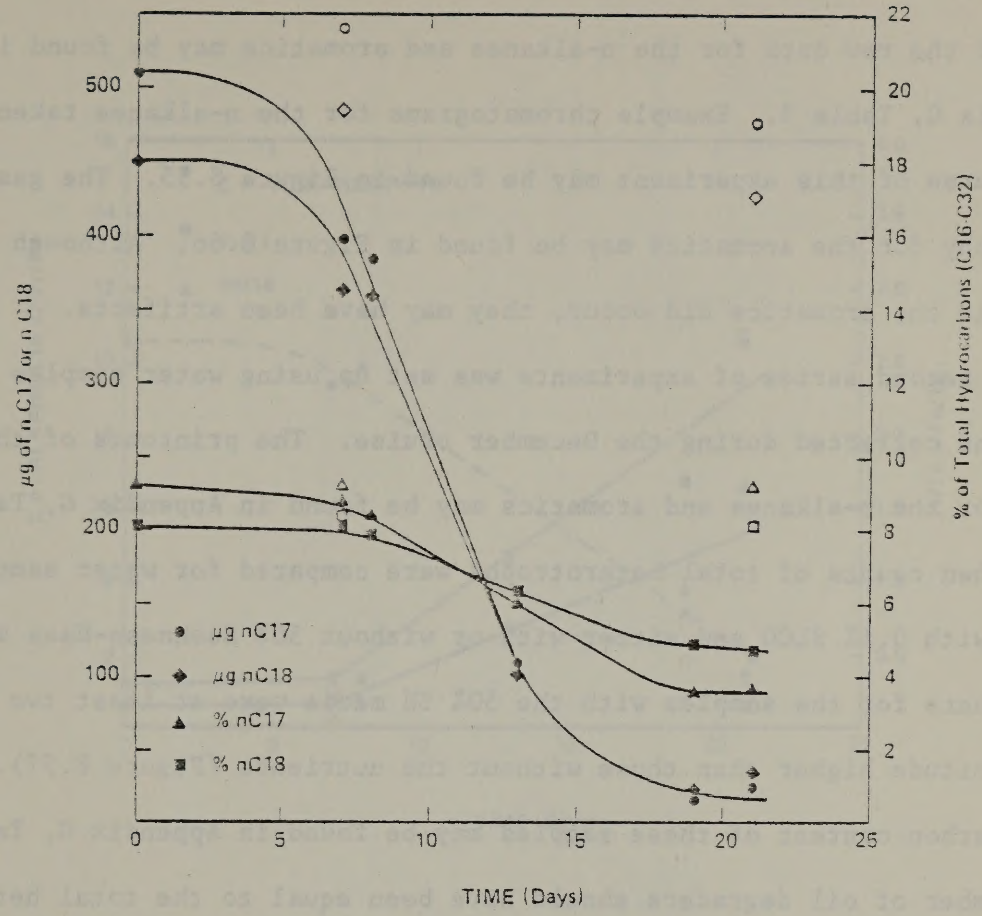


Figure 8.53 Utilization of Normal 17 and 18 Carbon Alkanes by Natural Bacterial Populations Presented as μg Remaining in Time and Also as a Percent in Time. Controls are Open Symbols.

metabolism of the n-alkanes and also show a disproportionate rate of metabolism of the n-alkanes and the branched alkanes (Figure 8.54). The printouts of the raw data for the n-alkanes and aromatics may be found in Appendix G, Table 3. Example chromatograms for the n-alkanes taken during the course of this experiment may be found in Figure 8.55. The gas chromatography for the aromatics may be found in Figure 8.56. Although differences in the aromatics did occur, they may have been artifacts.

A second series of experiments was set up using water samples for stations collected during the December cruise. The printouts of the raw data for the n-alkanes and aromatics may be found in Appendix G, Table 3.

When counts of total heterotrophs were compared for water samples grown with 0.1% SLCO and either with or without 50% Bushness-Haas media, the counts for the samples with the 50% BH media were at least two orders of magnitude higher than those without the nutrients (Figure 8.57). The hydrocarbon content of these samples may be found in Appendix G, Table 3. The number of oil degraders should have been equal to the total heterotrophs at this point of the experiments, but the experiment failed due to problems with the culture media.

Kinetics

Growth rates defined specifically as growth times (the time required for the bacteria to cause a change in optical density at 600 nm from 0.1 to 0.2) were measured for 15 strains of bacteria including 11 *Pseudomonas*, one Coryneform, one Group 4-E and two unidentified. Early growth experiments were carried out in 250 ml Erlenmeyer flasks using 2216 broth. The organism used, 3A'0.1 (1), was identified as a *Pseudomonas* in later

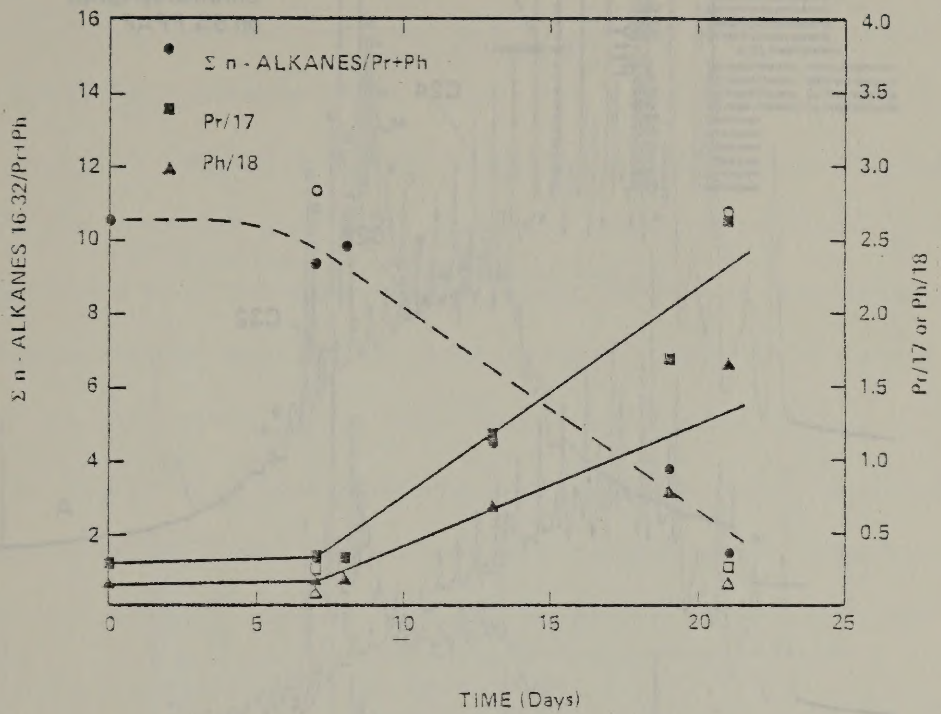


Figure 8.54 Ratios of Several Normal and Branched Alkanes Showing Disproportionate Utilization of the Hydrocarbons. Controls are Open Symbols.

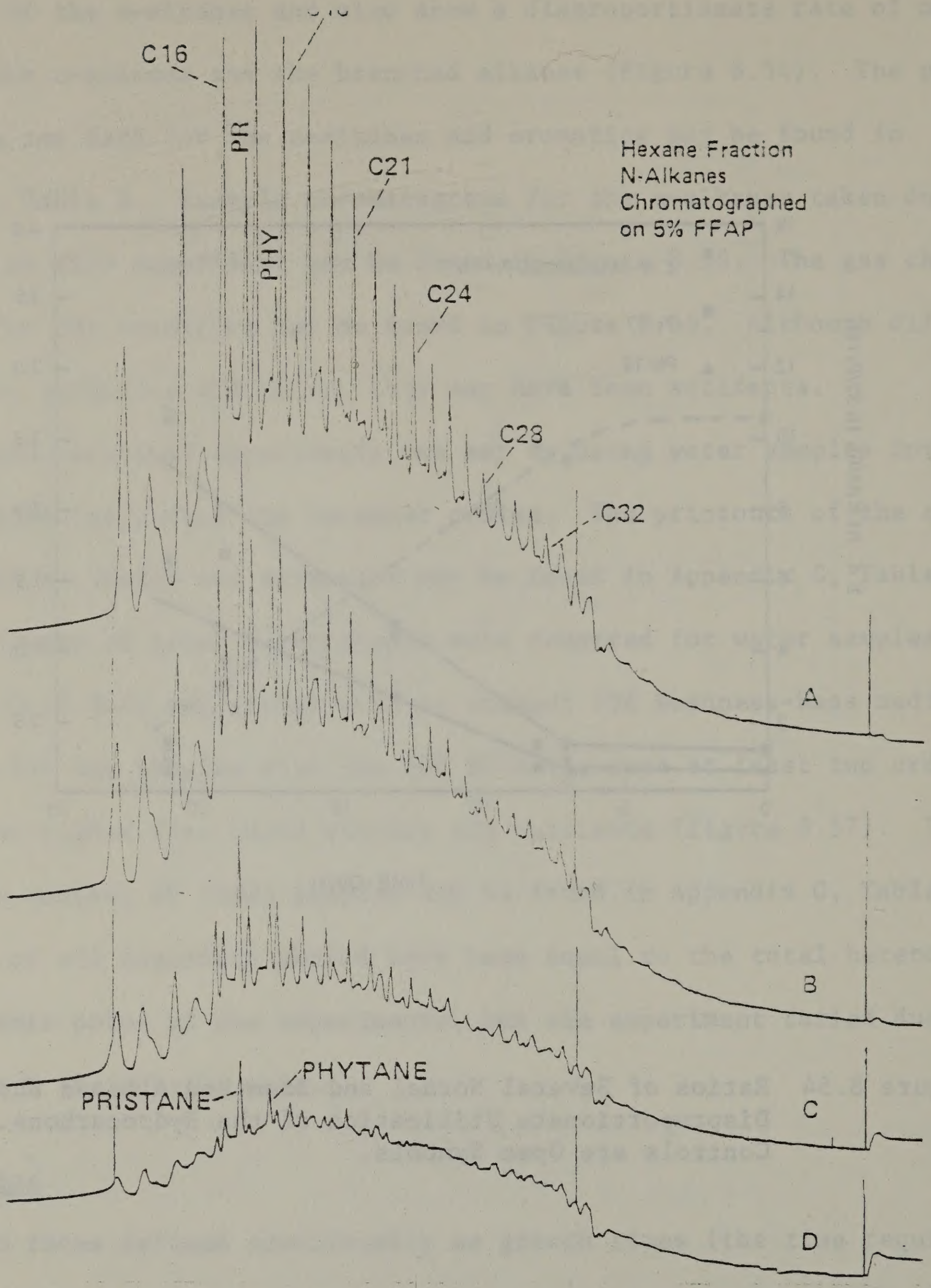
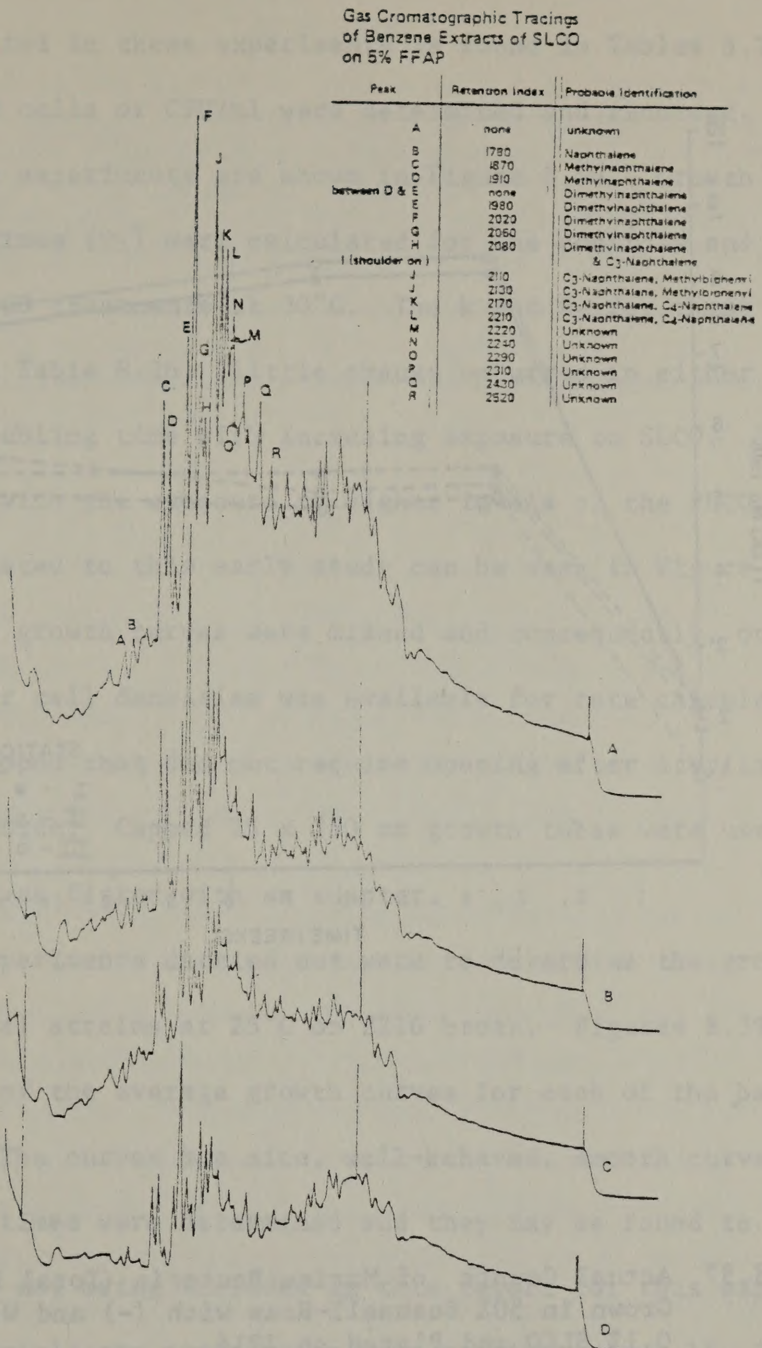


Figure 8.55 Example GC's of the Hexane Fractions From (A) Zero Time; (B) 7-day; (C) 13-day; and (D) 21-day Growth Culture of Natural Populations of Marine Bacteria Grown on SLCO. Weathering Controls are the Same as the Zero Time.



Gas Cromatographic Tracings of Benzene Extracts of SLCO on 5% FFAP

Peak	Retention Index	Probable Identification
A	none	unknown
B	1780	Naphthalene
C	1870	Methylnaphthalene
D	1910	Methylnaphthalene
between D & E	none	Dimethylnaphthalene
E	1980	Dimethylnaphthalene
F	2020	Dimethylnaphthalene
G	2060	Dimethylnaphthalene
H	2080	Dimethylnaphthalene
I (shoulder on)		& C_3-Naphthalene
J	2110	C_3-Naphthalene, Methylbiphenyl
J	2130	C_3-Naphthalene, Methylbiphenyl
K	2170	C_3-Naphthalene, C_4-Naphthalene
L	2210	C_3-Naphthalene, C_4-Naphthalene
M	2220	Unknown
N	2240	Unknown
O	2290	Unknown
P	2310	Unknown
Q	2430	Unknown
R	2520	Unknown

Figure 8.56 Example GC's of the Benzene Fraction from (A) Zero Time; (B) 7-day; (C) 13-day; and (D) 21-day Growth Cultures of Natural Populations of Marine Bacteria Grown on SLCO. Weathering Controls are the Same as the Zero Time.

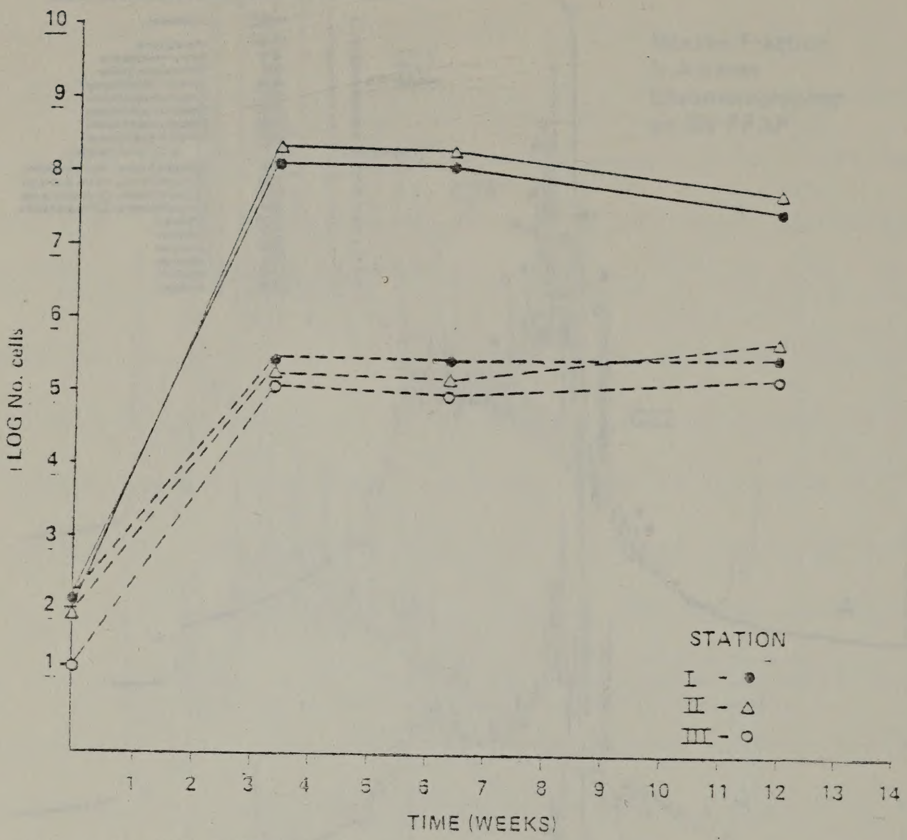


Figure 8.57 Actual Counts of Marine Bacteria (Total Heterotrophs) Grown in 50% Bushnell-Haas with (-) and Without (---) 0.1% SLCO and Plated on 2216.

studies. The effect of SLCO on this strain was investigated. Turbidimetric data collected in these experiments is found in Tables 8.22 through 8.25. Both OD and cells or CFU/ml were determined and recorded. Growth kinetics for these experiments are shown in Figure 8.58. Growth constants (k) and doubling times (D_2) were calculated for the controls and 0.06%, 0.33% and 0.67% SLCO treatments at 30°C. The k and D_2 for these experiments are found in Table 8.26. Little change occurred in either growth constant or the doubling time with incresing exposure to SLCO. Doubling time did decrease with the exposure to higher levels of the SLCO.

Problems related to this early study can be seen in Figure 8.58. The low end of the growth curves were missed and consequently, only limited data at higher cell densities was available for rate calculations. A system was developed that did not require opening after sterilization except for inoculation. Capped 25 x 250 mm growth tubes were used and the Spectronic 20 was fitted with an adapter.

The first experiments carried out were to determine the growth times for the 15 bacterial strains at 25°C on 2216 broth. Figures 8.59 through 8.63 are examples of the average growth curves for each of the bacterial strains at 25°C. The curves are nice, well-behaved, smooth curves. From these data, growth times were determined and they may be found in Table 8.27. Raw data is not being included in this report for this experiment.

Additional triplicate experiments were carried out at 15, 31, 41, 45 and 50°C. Growth times were determined and they may be found in Table 8.27 also. None of the strains grew at 50°C. The growth times for several strains may be found in Figure 8.64. These strains all showed a rapid increase in the growth time just above 40°C. The response below 40°C was for the most part a gradual increase in the growth time with decreasing temperature.

TABLE 8.22

GROWTH KINETICS FOR BACTERIAL STRAIN $3A^{1}0.1(1)$ ISOLATED FROM STATION 3, TRANSECT II, WINTER CRUISE.
CONTROL POPULATIONS WERE GROWN IN MARINE BROTH (2216 DIFCO), AT 30°C, IN A WATER BATH SHAKER, AT 100 OSCILLATIONS/MINUTE. POPULATIONS WERE ESTIMATED WITH A SPECTRONIC 20 AT 600 nm

	A			B			Ave.No. Cells/ml	Log_{10} Ave. Cells/ml
Time (Hrs)	OD	Cells/ml	Log_{10} Cells/ml	OD	Cells/ml	Log_{10} Cells/ml		
0.00	0.0	--	--	0.0	--	--	--	--
4.28	0.0	--	--	0.0	--	--	--	--
6.45	0.0	--	--	0.0	--	--	--	--
8.08	0.0	--	--	0.0	--	--	--	--
9.50	0.0	--	--	0.0	--	--	--	--
11.58	0.003	$1.1X10^{8}$	8.041	0.0	--	--	$1.1X10^{8}$*	8.041
14.08	0.100	$3.8X10^{9}$	9.580	0.110	$4.2X10^{9}$	9.623	$4.0X10^{9}$	9.602
15.08	0.163	$6.0X10^{9}$	9.778	0.200	$7.5X10^{9}$	9.875	$6.8X10^{9}$	9.833
16.37	0.202	$7.6X10^{9}$	9.881	0.320	$1.2X10^{10}$	10.079	$9.8X10^{9}$	9.981
17.38	0.312	$1.2X10^{10}$	10.079	0.417	$1.6X10^{10}$	10.195	$1.4X10^{10}$	10.146
18.33	0.385	$1.5X10^{10}$	10.176	0.495	$1.9X10^{10}$	10.279	$1.7X10^{10}$	10.230
24.28	0.58	$2.2X10^{10}$	10.342	0.69	$2.6X10^{10}$	10.415	$2.4X10^{10}$	10.380
27.53	0.66	$2.5X10^{10}$	10.398	0.70	$2.6X10^{10}$	10.415	$2.6X10^{10}$	10.415
30.03	0.74	$2.8X10^{10}$	10.447	0.72	$2.7X10^{10}$	10.432	$2.8X10^{10}$	10.447
31.92	0.77	$2.9X10^{10}$	10.462	0.74	$2.8X10^{10}$	10.447	$2.9X10^{10}$	10.462
34.00	0.83	$3.1X10^{10}$	10.491	0.80	$3.0X10^{10}$	10.477	$3.1X10^{10}$	10.491
37.22	0.90	$3.4X10^{10}$	10.531	0.88	$3.3X10^{10}$	10.519	$3.4X10^{10}$	10.531
39.08	0.98	$3.7X10^{10}$	10.563	0.99	$3.7X10^{10}$	10.568	$3.7X10^{10}$	10.568
49.90	1.15	$4.3X10^{10}$	10.633	1.15	$4.3X10^{10}$	10.633	$4.3X10^{10}$	10.633
55.32	1.14	$4.3X10^{10}$	10.633	1.19	$4.5X10^{10}$	10.653	$4.4X10^{10}$	10.643
57.32	1.14	$4.3X10^{10}$	10.633	1.19	$4.5X10^{10}$	10.653	$4.4X10^{10}$	10.643
58.58	1.18	$4.4X10^{10}$	10.643	1.19	$4.5X10^{10}$	10.653	$4.5X10^{10}$	10.643

-- Below detection limit * Not Averaged

TABLE 8.23

GROWTH KINETICS FOR BACTERIAL STRAIN $3A^1 0.1(1)$ ISOLATED FROM STATION 3, TRANSECT II, WINTER CRUISE.
TREATMENT POPULATIONS WERE GROWN IN MARINE BROTH WITH 0.06% STERILE SLCO ADDED AT ZERO TIME.
CULTURES WERE GROWN AT 30°C, IN A WATER BATH SHAKER, AT 100 OSCILATTIONS/MINUTE.
POPULATIONS WERE ESTIMATED WITH A SPECTRONIC 20 AT 600 mm

	A			B			Ave.No. Cells/ml	Log_{10} Ave. Cells/ml
Time (Hrs)	OD	Cells/ml	Log_{10} Cells/ml	OD	Cells/ml	Log_{10} Cells/ml		
0.00	0.00	--	--	0.00	--	--	--	--
4.28	0.00	--	--	0.00	--	--	--	--
6.45	0.00	--	--	0.00	--	--	--	--
8.08	0.00	--	--	0.00	--	--	--	--
9.50	0.00	--	--	0.002	$7.5X10^7$	7.875	$7.5X10^7$*	7.875*
11.58	0.00	--	--	0.011	$4.1X10^8$	8.613	$4.1X10^8$*	8.613*
14.08	0.085	$3.2X10^9$	9.505	0.055	$2.1X10^9$	9.322	$2.7X10^9$	9.431
15.08	0.170	$6.4X10^9$	9.806	0.160	$6.0X10^9$	9.778	$6.2X10^9$	9.792
16.37	0.215	$8.1X10^9$	9.908	0.190	$7.1X10^9$	9.851	$7.6X10^9$	9.881
17.38	0.355	$1.3X10^{10}$	10.114	0.310	$1.2X10^{10}$	10.079	$1.3X10^{10}$	10.114
18.33	0.420	$1.6X10^{10}$	10.204	0.405	$1.5X10^{10}$	10.176	$1.6X10^{10}$	10.204
24.28	0.62	$2.3X10^{10}$	10.362	0.61	$2.3X10^{10}$	10.362	$2.3X10^{10}$	10.362
27.53	0.69	$2.6X10^{10}$	10.415	0.69	$2.6X10^{10}$	10.415	$2.6X10^{10}$	10.415
30.03	0.75	$2.8X10^{10}$	10.447	0.77	$2.9X10^{10}$	10.462	$2.9X10^{10}$	10.462
31.92	0.82	$3.1X10^{10}$	10.491	0.84	$3.2X10^{10}$	10.505	$3.2X10^{10}$	10.505
34.00	0.93	$3.5X10^{10}$	10.544	0.95	$3.6X10^{10}$	10.556	$3.6X10^{10}$	10.556
37.22	0.95	$3.6X10^{10}$	10.556	0.98	$3.7X10^{10}$	10.568	$3.7X10^{10}$	10.568
39.08	0.98	$3.7X10^{10}$	10.568	0.99	$3.7X10^{10}$	10.568	$3.7X10^{10}$	10.568
49.90	1.2	$4.5X10^{10}$	10.653	1.10	$4.1X10^{10}$	10.613	$4.3X10^{10}$	10.633
55.32	1.2	$4.5X10^{10}$	10.653	1.10	$4.1X10^{10}$	10.613	$4.3X10^{10}$	10.633
57.25	1.2	$4.5X10^{10}$	10.653	1.10	$4.1X10^{10}$	10.613	$4.3X10^{10}$	10.633
58.58	1.2	$4.5X10^{10}$	10.653	1.00	$3.8X10^{10}$	10.580	$4.2X10^{10}$	10.623

-- Below detection limit

* Not averaged

TABLE 8.24

GROWTH KINETICS FOR BACTERIAL STRAIN $3A^1 0.1(1)$ ISOLATED FROM STATION 3, TRANSECT II, WINTER CRUISE.

TREATMENT POPULATIONS WERE GROWN IN MARINE BROTH WITH 0.33% STERILE SLCO ADDED AT ZERO TIME.

CULTURES WERE GROWN AT 30°C IN A WATER BATH SHAKER, AT 100 OSCILLATIONS/MINUTE.

POPULATIONS WERE ESTIMATED WITH A SPECTRONIC 20 AT 600 mm

	A			B			Ave.No. Cells/ ml	Log_{10} Ave Cells/ml
Time (Hrs)	OD	Cells/ml	Log_{10} Cells/ml	OD	Cells/ml	Log_{10} Cells/ml		
0.00	0.00	--	--	0.00	--	--	--	--
4.28	0.00	--	--	0.00	--	--	--	--
6.45	0.00	--	--	0.00	--	--	--	--
8.08	0.00	--	--	0.00	--	--	--	--
9.50	0.00	--	--	0.00	--	--	--	--
11.58	0.00	--	--	0.00	--	--	--	--
14.08	0.10	$3.7X10^9$	9.568	0.00	--	--	$3.7X10^9$*	9.568*
15.08	0.115	$4.3X10^9$	9.633	0.07	$2.6X10^9$	9.415	$3.5X10^9$	9.544
16.37	0.175	$6.6X10^9$	9.820	0.165	$6.2X10^9$	9.792	$6.4X10^9$	9.806
17.38	0.320	$1.2X10^9$	10.079	0.280	$1.1X10^{10}$	10.041	$1.2X10^{10}$	10.079
18.33	0.410	$1.5X10^{10}$	10.176	0.335	$1.3X10^{10}$	10.114	$1.4X10^{10}$	10.146
24.28	0.62	$2.3X10^{10}$	10.362	0.56	$2.1X10^{10}$	10.322	$2.2X10^{10}$	10.342
27.53	0.69	$2.6X10^{10}$	10.415	0.64	$2.4X10^{10}$	10.380	$2.5X10^{10}$	10.398
30.03	0.72	$2.7X10^{10}$	10.431	0.67	$2.5X10^{10}$	10.398	$2.6X10^{10}$	10.415
31.92	0.73	$2.7X10^{10}$	10.431	0.72	$2.7X10^{10}$	10.431	$2.7X10^{10}$	10.431
34.00	0.89	$3.3X10^{10}$	10.519	0.85	$3.2X10^{10}$	10.505	$3.3X10^{10}$	10.519
37.22	0.96	$3.6X10^{10}$	10.556	0.93	$3.5X10^{10}$	10.544	$3.6X10^{10}$	10.556
39.08	0.99	$3.7X10^{10}$	10.568	0.95	$3.6X10^{10}$	10.556	$3.7X10^{10}$	10.568
49.90	1.15	$4.3X10^{10}$	10.633	1.03	$3.9X10^{10}$	10.591	$4.1X10^{10}$	10.613
55.32	1.15	$4.3X10^{10}$	10.633	1.00	$3.8X10^{10}$	10.580	$4.1X10^{10}$	10.613
57.25	1.11	$4.2X10^{10}$	10.623	1.00	$3.8X10^{10}$	10.580	$4.0X10^{10}$	10.602
58.58	1.10	$4.2X10^{10}$	10.623	1.00	$3.8X10^{10}$	10.580	$4.0X10^{10}$	10.602

-- Below Detection Limit * Not Averaged

TABLE 8.25

GROWTH KINETICS FOR BACTERIAL STRAIN 3A^{1}0.1(1) ISOLATED FROM STATION 3, TRANSECT II, WINTER CRUISE.
TREATMENT POPULATIONS WERE GROWN IN MARINE BROTH WITH 0.67% STERILE SLCO ADDED AT ZERO TIME.
CULTURES WERE GROWN AT 30°C IN A WATER BATH SHAKER, AT 100 OSCILLATIONS/MINUTE.
POPULATIONS WERE ESTIMATED WITH A SPECTRONIC 20 AT 600 mm.

Time (hrs)	A			B			Ave. No. Cells/ml	Log_{10} Ave Cells/ml
	OD	Cells/ml	Log_{10} Cells/ml	OD	Cells/ml	Log_{10} Cells/ml		
0.00	0.00	--	--	0.00	--	--	--	--
4.28	0.00	--	--	0.00	--	--	--	--
6.45	0.00	--	--	0.00	--	--	--	--
8.08	0.00	--	--	0.00	--	--	--	--
9.50	0.00	--	--	0.00	--	--	--	--
11.58	0.00	--	--	0.00	--	--	--	--
14.08	0.00	--	--	0.00	--	--	--	--
15.08	0.090	$3.4x10^9$	9.531	0.080	$3.0x10^9$	9.477	$3.2x10^9$	9.505
16.37	0.165	$6.2x10^9$	9.792	0.150	$5.6x10^9$	9.748	$5.9x10^9$	9.770
17.38	0.275	$1.0x10^{10}$	10.000	0.240	$9.0x10^9$	9.954	$1.0x10^{10}$	10.000
18.33	0.355	$1.3x10^{10}$	10.114	0.292	$1.1x10^{10}$	10.041	$1.2x10^{10}$	10.079
24.28	0.460	$1.7x10^{10}$	10.230	0.54	$2.0x10^{10}$	10.301	$1.9x10^{10}$	10.279
27.53	0.66	$2.5x10^{10}$	10.398	0.63	$2.4x10^{10}$	10.380	$2.5x10^{10}$	10.398
30.03	0.67	$2.5x10^{10}$	10.398	0.68	$2.6x10^{10}$	10.415	$2.6x10^{10}$	10.415
31.92	0.71	$2.7x10^{10}$	10.431	0.70	$2.6x10^{10}$	10.415	$2.7x10^{10}$	10.431
34.00	0.85	$3.2x10^{10}$	10.505	0.81	$3.0x10^{10}$	10.477	$3.1x10^{10}$	10.491
37.22	0.91	$3.4x10^{10}$	10.531	0.89	$3.3x10^{10}$	10.519	$3.4x10^{10}$	10.531
39.08	0.94	$3.5x10^{10}$	10.544	0.94	$3.5x10^{10}$	10.544	$3.5x10^{10}$	10.544
49.90	1.15	$4.3x10^{10}$	10.633	1.15	$4.3x10^{10}$	10.633	$4.3x10^{10}$	10.633
55.32	1.13	$4.2x10^{10}$	10.623	1.12	$4.2x10^{10}$	10.623	$4.2x10^{10}$	10.623
57.25	1.12	$4.2x10^{10}$	10.623	1.19	$4.5x10^{10}$	10.653	$4.4x10^{10}$	10.643
58.58	1.12	$4.2x10^{10}$	10.623	1.19	$4.5x10^{10}$	10.653	$4.4x10^{10}$	10.643

-- Below detection limit

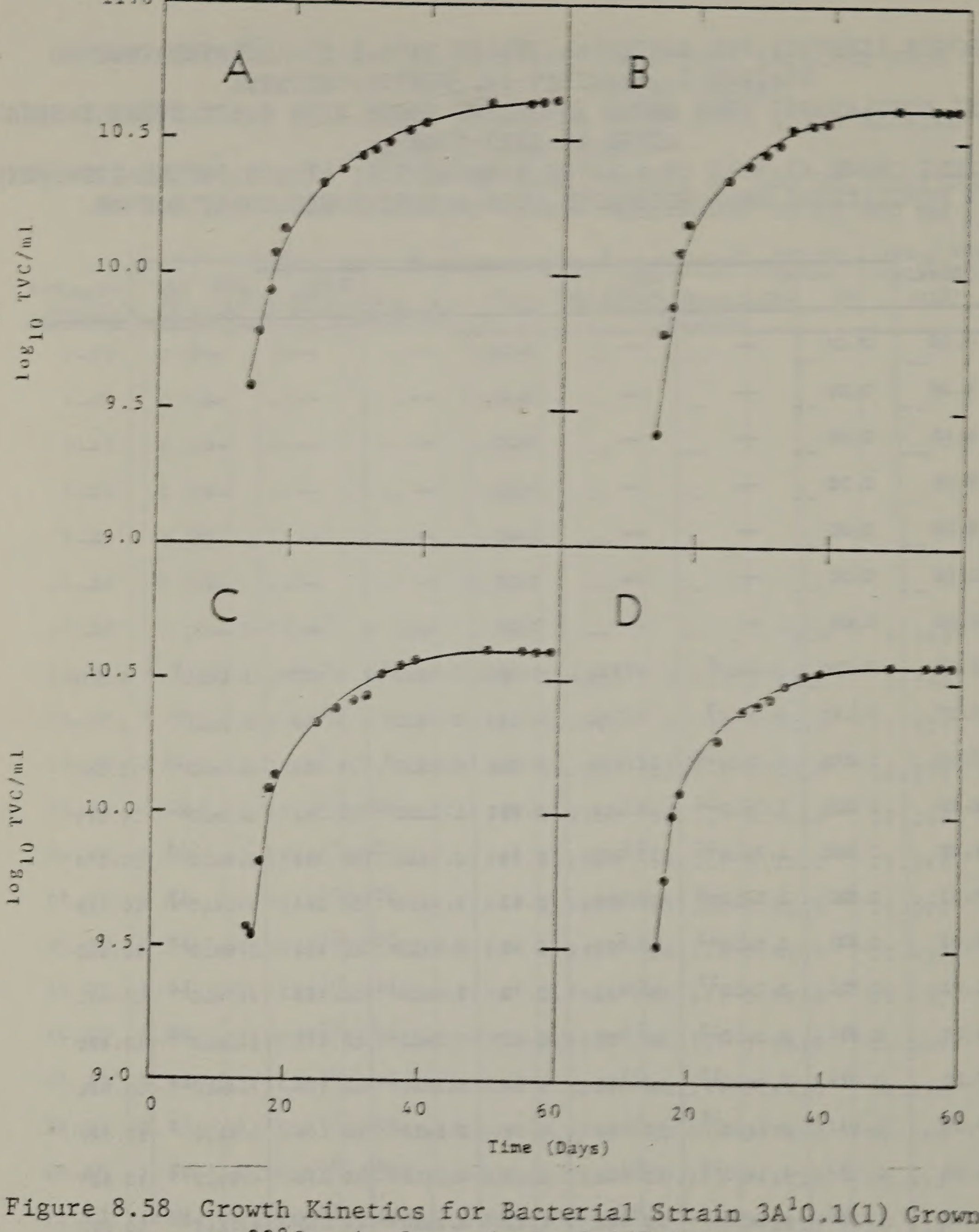


Figure 8.58 Growth Kinetics for Bacterial Strain $3A^10.1(1)$ Grown at 30°C. A, Control; B, 0.06% SLCO; C, 0.33% SLCO; and D, 0.67% SLCO.

TABLE 8.26

GROWTH KINETICS (k) AND DOUBLING TIMES (D_2) FOR STRAINS 3A^{1}0.1(1) GROWN AT 30°C AND TREATED WITH SEVERAL CONCENTRATIONS OF SLCO.

Oil Concentration	k			D_2		
	A	B	Ave.	A	B	Ave.
0.00%	0.50*	0.44	0.47	2.0**	2.26	2.13
0.06%	0.66	1.00	0.83	1.51	1.00	1.26
0.33%	0.66	0.89	0.78	1.51	1.13	1.32
0.67%	0.91	0.91	0.91	1.09	1.09	1.09

* Doublings/hr

** Time of doubling in hrs.

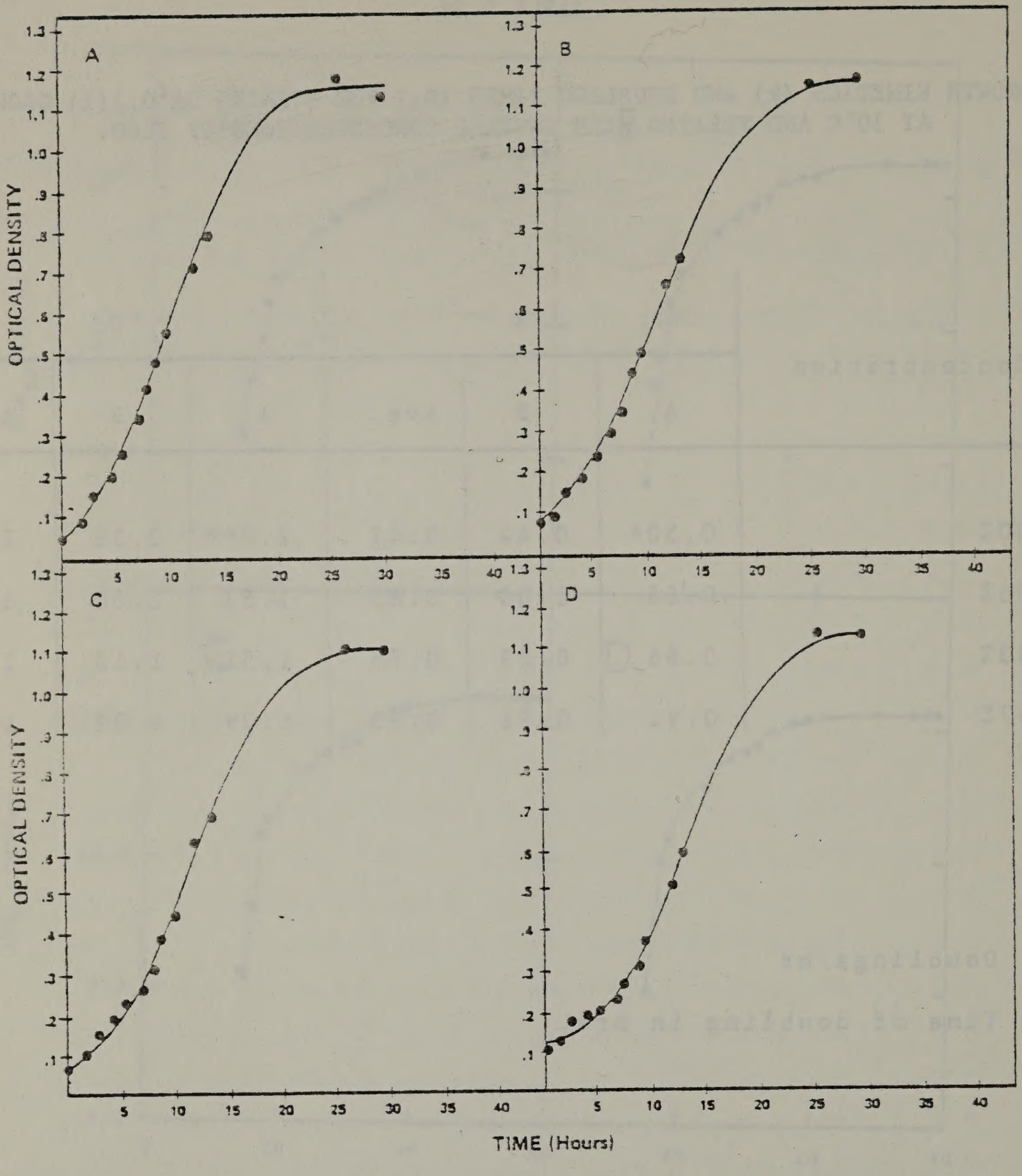


Figure 8.59 Growth Curves for Four Strains of Marine Bacteria. A. *Pseudomonas* (112'01511-1), B. *Pseudomonas*(11201511-8), C. Group 4-E (11201511-7C), D. *Pseudomonas* (112'01511-5C).

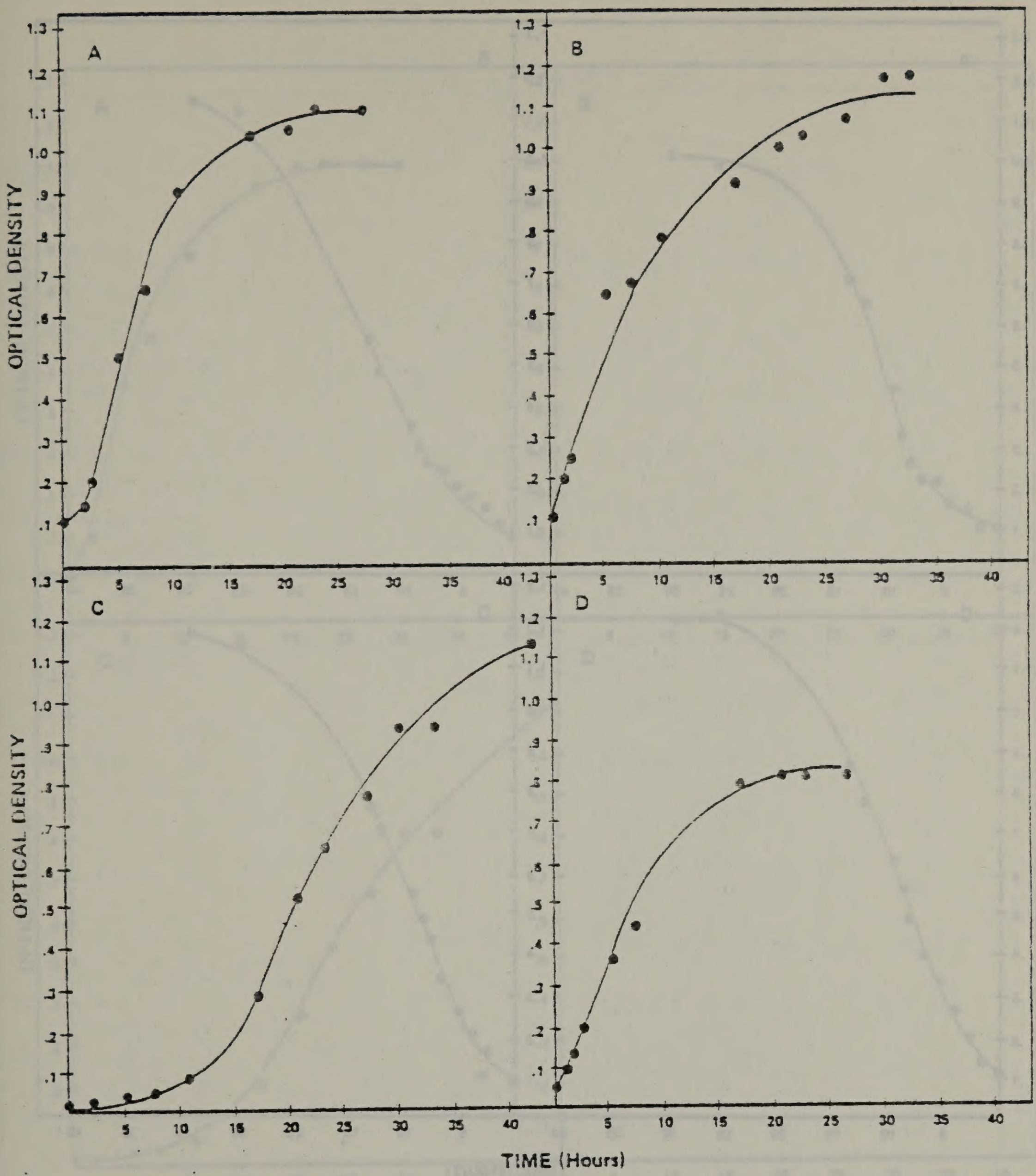


Figure 8.60 Growth Curves for Four Strains of Marine Bacteria. A. Unknown (13201517-1), B. *Pseudomonas* (131'01517-1), C. *Pseudomonas* (11201511-6A), D. *Pseudomonas* (11201511-7B).

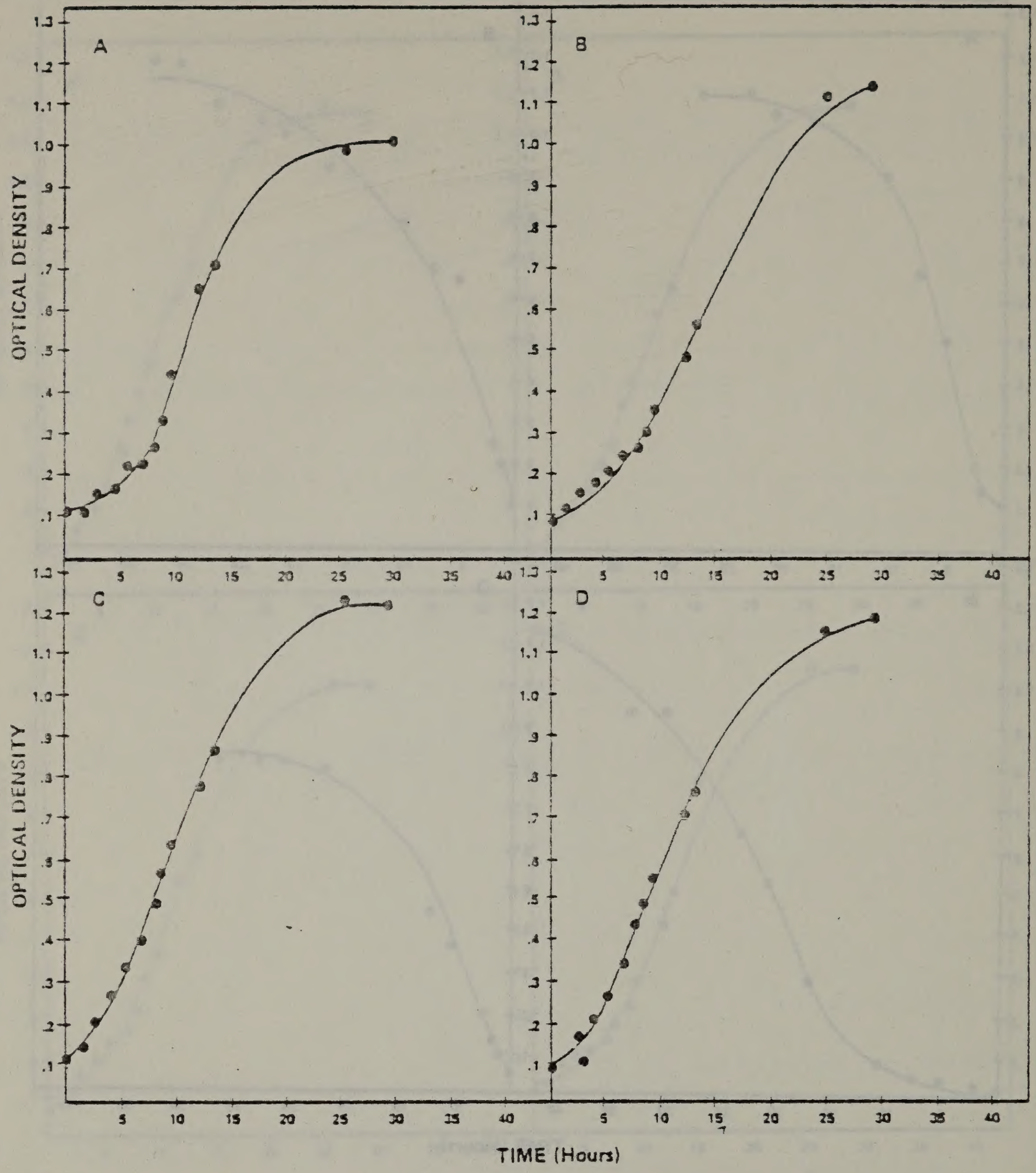


Figure 8.61 Growth Curves for Four Strains of Marine Bacteria.
A. Coryneform (611118067), B. *Pseudomonas* (112'01511-2A),
C. *Pseudomonas* (111'02516-3), D. *Pseudomonas* (11102516-7)

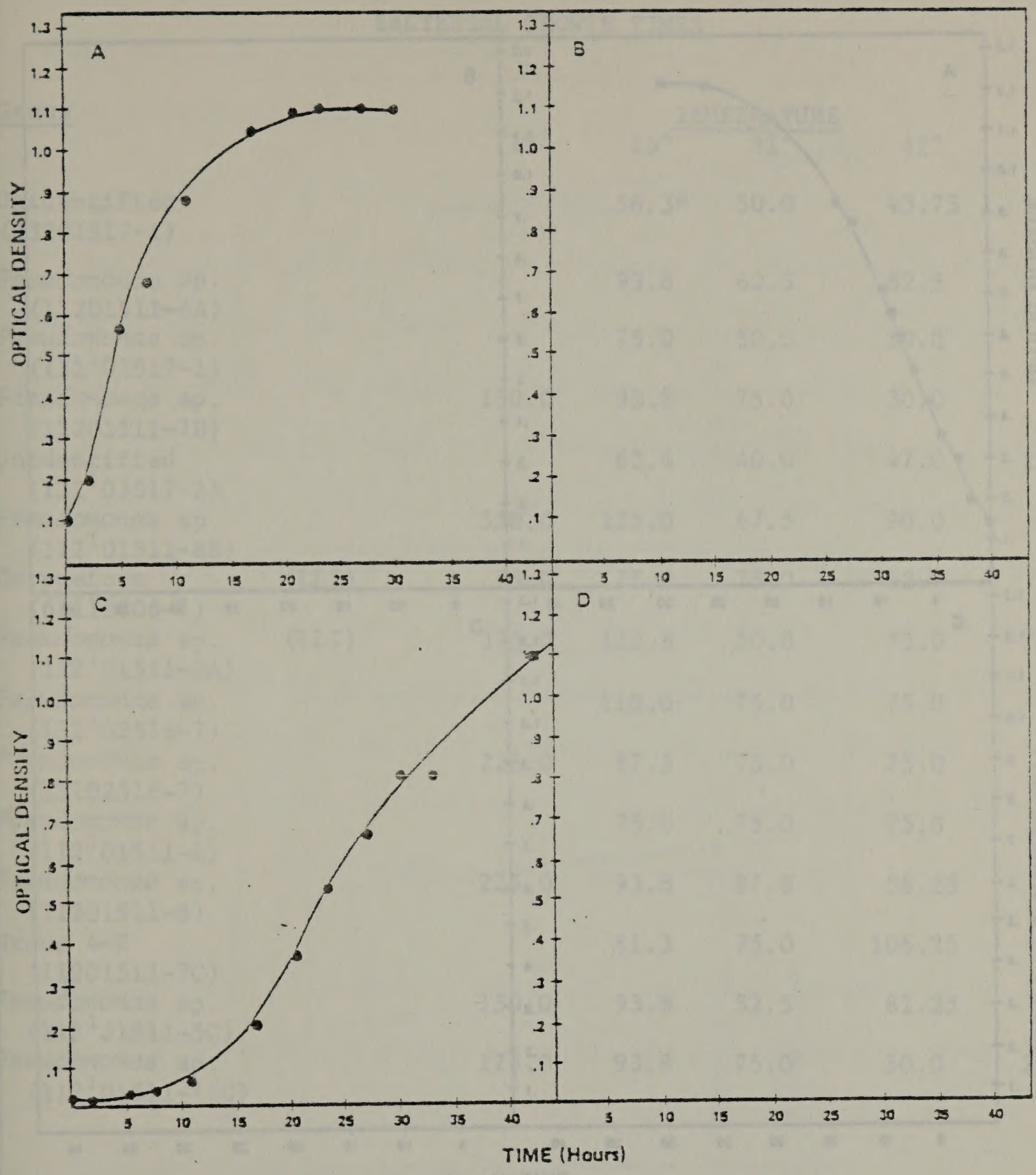


Figure 8.62 Growth Curves for Two Strains of Marine Bacteria. A. Unidentified (132'03517-2), C. *Pseudomonas* (112'01511-8B).

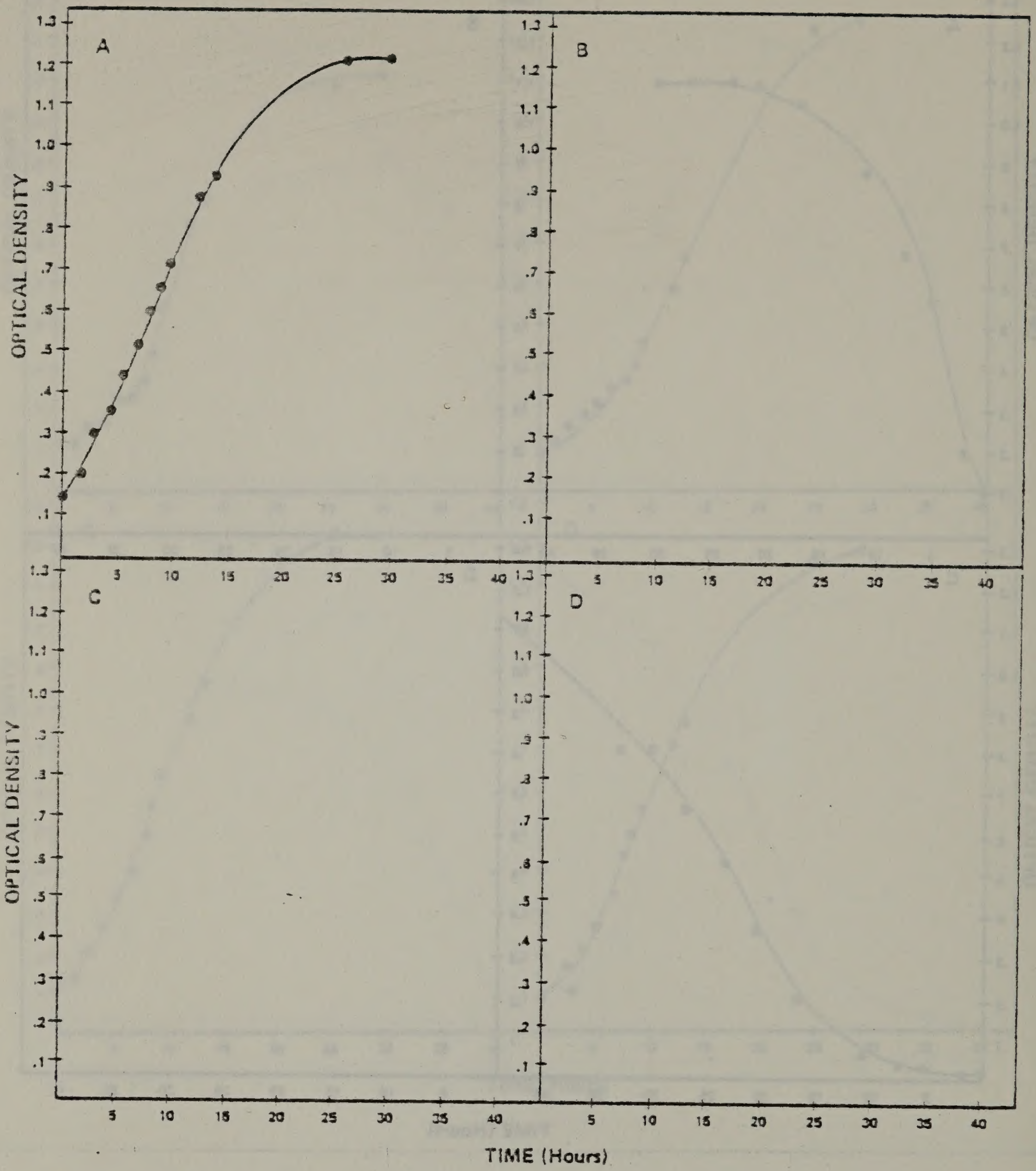


Figure 8.63 Growth Curve for One Strain of Marine Bacteria.
A. *Pseudomonas* (112'015111-11C).

TABLE 8.27

BACTERIAL GROWTH TIMES

Genus				TEMPERATURE		
		15°	25°	31°	41°	45°
Unidentified (13201517-1)			56.3*	50.0	43.75	187.5
Pseudomonas sp. (11201511-6A)			93.8	62.5	82.5	
Pseudomonas sp. (131^{1}01517-1)			75.0	50.0	30.0	
Pseudomonas sp. (11201511-7B)		150.0	93.8	75.0	30.0	75.0
Unidentified (132^{1}03517-2)			62.4	40.0	47.0	225.0
Pseudomonas sp. (112^{1}01511-8B)		550.0	125.0	67.5	90.0	
Coryneform (61111806-7)	(LLT)	75.0	75.0	75.0	62.7	93.75
Pseudomonas sp. (112^{1}01511-2A)	(LLT)	375.0	112.8	50.0	75.0	787.5
Pseudomonas sp. (111^{1}02516-7)			110.0	75.0	75.0	
Pseudomonas sp. (11102516-7)		225.0	87.5	75.0	75.0	
Pseudomonas sp. (112^{1}01511-1)			75.0	75.0	75.0	
Pseudomonas sp. (11201511-8)		225.0	93.8	87.8	56.25	
Group 4-E (11201511-7C)			81.3	75.0	106.25	
Pseudomonas sp. (112^{1}01511-5C)		150.0	93.8	52.5	81.25	
Pseudomonas sp. (112^{1}01511-11C)		175.0	93.8	75.0	50.0	225.0

*Growth Time in Minutes
LLT=Long Lag Time

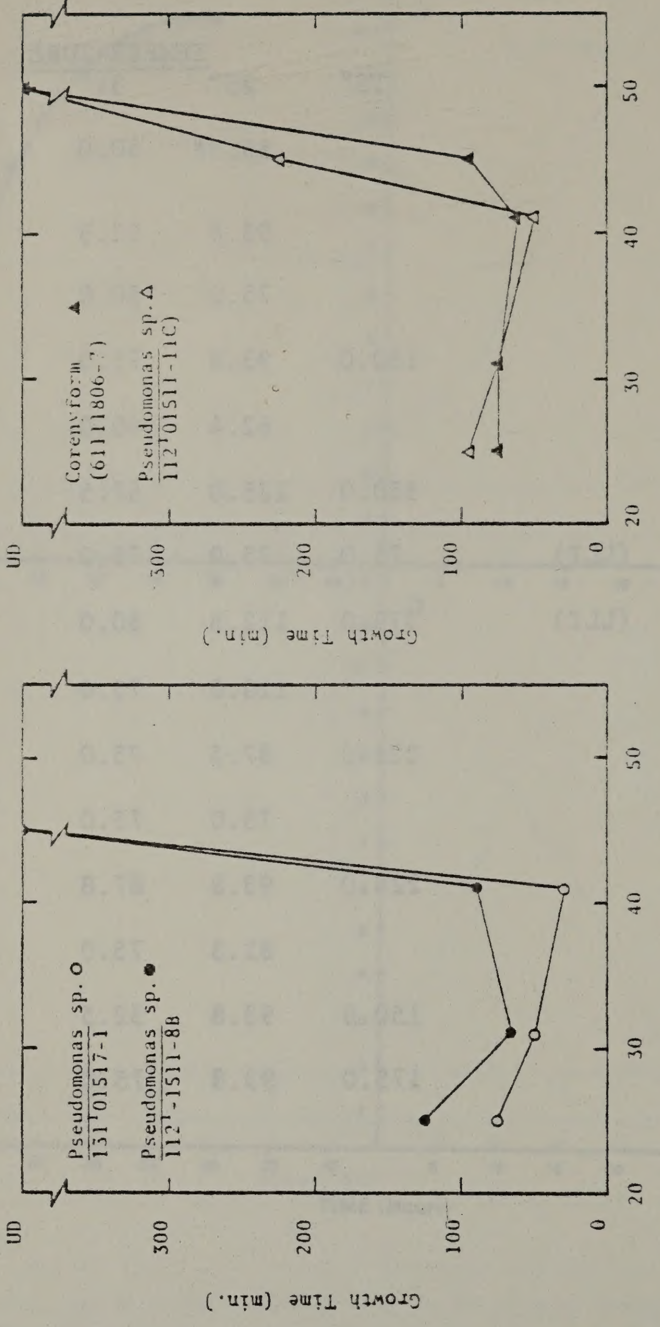


Figure 8.64 The Effect of Temperature on Bacterial Kinetics.

Another series of experiments testing the interaction of SLCO and temperature was carried out. Oil concentrations used were 0.0, 0.1, 0.5 and 1.0. Temperatures tested were 25, 31, and 41°C. Little change in the growth times occurred.

DISCUSSION

The water column bacteriology studies on the 27 samples collected during 1977 provided data, which although limited by only one year's investigations, provided some insight into a preliminary overall picture of the distribution and abundance of the heterotrophic and hydrocarbonoclastic bacterial genera occurring in the water column at the one meter depth. Results of these investigations of the bacterial populations in the water column along Transect II have indicated that relatively few genera make up the predominants of the stations studied. Only 14 genera of heterotrophs were identified, and of this group, three genera (*Bacillus*, *Planococcus*, and *Xanthomonas*) only occurred once. The luminescent *Photobacterium* strains were isolated only in April at Stations 2 and 3 and at Station 1 in June.

Pseudomonas, *Alcaligenes*, *Aeromonas*, and *Vibrio* appeared to be the predominants for both the heterotrophs and the oil degraders. These organisms are all Gram negative, aerobic rods, and apparently have many representatives which can degrade the crude oil.

The microbial population of the water column was relatively small when considered with those populations of the sediments. However, the 5. x 10^2 to 1.55 x 10^5 cells per liter for heterotrophs was similar to values for the water column reported by other investigators (Roubal and Atlas, 1978).

Early issues dealt with the kinds of media to use in the enumeration of various types of bacteria. The medium for the oil degraders is still a problem. The choices were salt water, nutrient agar, or an inert solid medium, such as silica gel, which was finally used. Early experiments indicated that the SGO medium should be modified to contain 0.5 g/ℓ $FeCl_3$. With this modification came reduced variability and increased reliability.

Bacterial succession enumeration work proceeded quite well. Increases in populations were found to occur within a week of bringing the samples into the laboratory. A preliminary experiment indicated this increase occurred within 24 h. Other investigators have found similar trends for marine bacteria. Apparently, the increased surface area provided by the container available to the organisms stimulated this burst of growth.

Seasonal trends have been reported for marine bacteria in the past, and seasonal trends have been reported for certain chemical factors in the South Texas Outer Continental Shelf (STOCS) area, but we did not find any distinct temporal patterns in our enumeration data for succession studies for any of the sampling sites. Further statistical analyses need to be completed before the final answer to this question is given. Definite differences occurred between the populations of total heterotrophs and the oil degraders at each site and time, but the site differences were not that great or constant. Further analyses will also be required here.

Trends were noted with the bacterial numbers for both groups or organisms. Successional populations in closed systems with or without SLCO increased to a maximum level very quickly and then did not change appreciably thereafter. This pattern appeared consistently and was probably due to nutrient limitation.

Species trends during successional studies were clouded. Differences with or without oil were very slight. The genera represented at the start of the study were not always present at the end. Dominants at the beginning were not dominant at the end. Genera that were minor components or not even detected at the beginning sometimes became dominant at the end. These trends were expected for the samples.

The strains that were found during the succession studies were common genera that have been reported from many marine environments. One strain, a minor component, was identified as a pathogen, but it may have been a contaminant. Becuase of a problem with one of our diagnostic tests, which was not discovered until the end of the contract period, we have not been able to separate several strains of four genera. The problem has been worked out, but unfortunately, not in time to be included in this report.

Studies with raw or natural Gulf of Mexico water containing indigenous bacterial populations did not show any hydrocarbon metabolism or degradation. Slight changes in sample hydrocarbon content occurred, but changes were as great or greater in the controls. The addition of complete mineral media or NO_3, PO_4, and Fe caused the metabolism of 75 to 95% of the saturated hydrocarbons. Aromatics seemed to be removed as well, but this indication appeared to be an artifact. Other investigators have reported similar results from other oceanic or marine areas.

Kinetic investigations showed that all isolates tested were mesothermal. They had optimum growth times between 30 and 40°C. If other isolates from winter cruises or deep waters had been used some psycrophiles may have been encountered. Strains tested showed a rapid increase in growth time just above the optimum. This interesting "threshold" has been reported for other strains of bacteria. Double stressed bacteria, which were stressed with temperature and oil, did not show any apparent change in their growth times. Further statistical treatment will be required for the substantiation of the above.

In order to have a more meaningful set of data for the microorganisms of the Western Gulf of Mexico, it will be necessary to continue studies similar to these now being reported, for an additional two or three years. This would provide sufficient data for determining if the predominants and populations in this initial study are the most likely to occur. In addition, a more accurate assessment of the influence of water temperature and salinity on microbial growth and species distribution would have to be made.

In order to study the effects of other environmental factors on the bacterial populations of the water column, one should attempt to correlate the microbiological data with factors known to influence bacterial growth and metabolism. Some of these factors to be included are nutrients, total organic carbon, oxygen, temperature, salinity, trace metals (such as copper, magnesium, zinc, mercury, cadmium and manganese), phytoplankton, sediment type and texture, wave action, nitrates, and phosphates. Another aspect of the study which should be considered is that of collection time. Little information is available as to possible variations in microbial populations collected at different times of the day.

In order to more accurately assess the oil degradation potential of the hydrocarbonoclastic bacteria, additional degradation rate studies and fate and effects of oil studies should be conducted. Pure culture studies and mixed culture studies in this area need to be expanded if a more accurate estimate of the oil degradation potential is to be made.

CONCLUSIONS

1. Marine bacteria of the water column are relatively few in number when compared with those in the sediments. Heterotrophic bacteria ranged from 5.0×10^2 to 1.55×10^5 cells per liter, while hydrocarbonoclastic bacteria ranged from 0 to 12.9 cells per liter.

2. The percentage of oil degraders ranged from 0 to 2.05%, but the values were usually significantly less than 0.5%.

3. Heterotrophic populations exhibited wider diversity in June and July, but heterotrophic populations were usually much lower in July.

4. Hydrocarbonoclastic populations peaked in July and August, thereby resulting in increases in percentage of oil degraders.

5. A total of 1,905 marine bacteria were isolated from the water column samples and 1,631 generic identifications were completed. Fourteen (14) genera were represented in these isolates. *Acinetobacter, Aeromonas, Pseudomonas,* and *Vibrio* were the predominant genera for both the heterotrophs and the hydrocarbonoclastic bacteria.

6. *Pseudomonas* was the only genus represented in all 27 water column samples.

7. *Acinetobacter, Aeromonas, Pseudomonas,* and *Vibrio* accounted for 92.8% of all the heterotrophs and 88.1% of all the oil degraders.

8. The greatest generic diversity occurred at Station 3/II in April with eight genera represented. Usually only three or four genera represented the populations in the water column samples.

9. Seasonal fluctuations in heterotrophs were most prevalent at Stations 1/II and 3/II, while microbial populations at Station 2/II appeared to be more stable.

10. Seasonal fluctuations were observed between stations. The winter season exhibited offshore decreases in populations. This was least apparent in the fall.

11. Temperature fluctuations appeared to have had more significant effects on bacterial populations than did the salinity variations. Optimum temperature range for the marine organisms was from 23 to 26°C.

12. Pure culture and mixed culture studies on the effects of SLCO indicate that concentrations of 0.5% or less increased the growth rate after four weeks, following a decrease after the first 7 to 14 days of incubation.

13. The relationship of salinity should be more effectively explored, since many marine isolates demonstrated sensitivities, or tolerances, to several ranges of salinity.

14. The major genera of bacteria found in the successional studies were *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, and *Coryneforms*.

15. The presence of 0.1% or 0.5% SLCO did not seem to effect the number of bacteria present during the successional studies.

16. In some cases, the number of genera present in the succession cultures decreased with increasing time in the laboratory.

17. The presence of SLCO did not seem to effect the course of succession.

18. Hydrocarbon analyses and turbidimetric analyses indicated that an insignificant amount of SLCO was metabolized during the successional stages.

19. Oil degradation rates for Gulf of Mexico water tested were insignificant or essentially zero unless minimal nutrients were added to the water samples.

20. Additions of nitrates, phosphates, iron, or a compliment of mineral salts to the medium significantly enhanced hydrocarbon metabolism.

21. Kinetic studies indicated that optimum temperatures for growth of the bacterial isolates was 31-41°C.

22. The addition of SLCO did not alter growth time of selected strains of marine bacteria.

LITERATURE CITED

Atlas, R. M., and B. Busdosh. 1976. Microbial degradation of petroleum in the Arctic. *In* Proc. Third Internatl. Biodegradation Symposium. J. M. Sharpley and A. M. Kaplan (eds.), Applied Sci. Publ. London, pp 79-87.

Baumann, P., L. Baumann, and M. Mandel. 1971a. Taxonomy of marine bacteria: The Genus *Benecka*. J. Bacteriol. 107:268-294.

Baumann, L., P. Baumann, M. Mandel, and R. D. Allen. 1971b. Taxonomy of aerobic marine Eubacteria. J. Bacteriol. 110:402-429.

Bergey's Manual of Determinative Bacteriology. 1974. Eighth Edition. Williams and Wilkins Press, Baltimore.

Colwell, R. R. 1970. Polyphasic taxonomy of the Genus *Vibrio*: Numerical Taxonomy of the *Vibrio* cholerae, *Vibrio parahaemolyticus*, and related *Vibrio* species. J. Bacteriol. 104:410-433.

Dibble, J. T., and R. Bartha. 1976. Effects of iron on the biodegradation of petroleum in seawater. Appl. Envt. Microbiol. 31:544-550.

Floodgate, G. D., and P. R. Hayes. 1963. The Adansonian taxonomy of some yellow pigmented bacteria. J. Gen. Microbiol. 30:237-244.

Hayes, P. R. 1963. Studies on marine *Flavobacterium*. J. Gen. Microbiol. 30:1-19.

Horowitz, A., D. Gutnick, and E. Rosenberg. 1975. Sequential growth of bacteria on crude oil. Appl. Microbiol. 30:10-19.

Kator, H., C. H. Oppenheimer, and R. J. Miget. 1971. Microbial degradation of a Louisiana crude oil in closed flasks and under simulated field conditions. *In* Amer. Petrol. Inst/EPA/USCG Conf. Prevention and Control of Oil Spills. Amer. Pet. Inst. Washington, D. C. pp 287-296.

Lovelace, T. E., and R. R. Colwell. 1968. A multi-point inoculator for petri dishes. Appl. Micro. 16:944-945.

Miget, R. J., C. H. Oppenheimer, H. I. Kator, and D. A. LaRock. 1969. Microbial degradation of normal paraffin hydrocarbons in crude oil. Proc. Joint Conf. on Prevention and Control of Oil Spills. Amer. Pet. Inst. Washington, D. C. pp 327-331.

Mulkins-Phillips, G. J., and J. E. Stewart. 1974. Effects of environmental parameters on bacterial degradation of Bunker C oil, crude oils, and hydrocarbons. Appl. Microbiol. 28:915-922.

Prichard, P. H., R. M. Ventullo, and J. M. Suflita. 1976. The microbial degradation of diesel oil in multistage continuous culture systems.

In Proc. Third Internatl. Biodegradation Symp. J. M. Sharpley and A. M. Kaplan (eds.), Applied Sci. Publ. London, pp 67-78.

Roubal, G., and R. M. Atlas. 1978. Distribution of hydrocarbon utilizing microorganisms and hydrocarbon biodegradation potentials in Alaskan Continental Shelf Areas. Appl. Envtl. Microbiol. 35:897-905.

Soli, G., and E. M. Bens. 1972. Bacteria which attack petroleum hydrocarbon in a saline medium. Biotech. Bioeng. 14:319-330.

Stanier, R. Y., N. J. Palleroni, and D. Doudoroff. 1966. The aerobic pseudomondad: a taxonomic study. J. Gen. Microbiol. 43:159-271.

Walker, J. D., and R. R. Colwell. 1976a. Enumeration of petroleum-degrading microorganisms. Appl. Envrtl. Microbiol. 31:198-207.

____________. 1976b. Petroleum: degradation by estuarine microorganisms. *In* Proc. Third Internatl. Biodegradation Symp. J. M. Sharpley and A. M. Kaplan (eds). Appl. Sci. Publ. London, pp. 197-204.

CHAPTER NINE

WATER COLUMN AND BENTHIC MICROBIOLOGY-MYCOLOGY

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ABSTRACT

Water samples were collected at three stations during six monthly cruises. Bottom sediment samples were collected at six stations during three seasonal cruises. Sediments were diluted and water samples concentrated before plating on hydrocarbonoclastic selective and nonselective media for fungal enumeration. Sediment and water samples were overlaid with crude oil and sampled periodically to determine the effects of oil on the fungi. Upon termination of the experiments, the alkane and aromatic fractions of residual oils were analyzed to evaluate the degradation potential of each sample and the preferential degradation of individual oil components. Similar studies were conducted on fungi in pure culture. Several hundred isolates were tested for their ability to grow on crude oil as a sole source of carbon and energy.

The numbers of total fungi in the water column were uniformly distributed and much more abundant in spring than later in the year, but the percentage of oil-degraders increased offshore and throughout the year. Total benthic fungi increased from spring to fall. Fungal *n*-alkane degradation potentials of benthic and water samples tended to decrease offshore across the shelf especially during the summer. Degradation was greatly increased in the presence of river outwash. Intermediate length *n*-alkanes were preferentially utilized by fungi but isoalkanes were recalcitrant. Crude oil was initially toxic to the petroleum-degrading fungi but in general served to stimulate fungal growth after a period of one to several weeks.

INTRODUCTION

The objective of the 1977 Mycology Project was three-fold: 1) to describe the spatial and temporal variation in the abundance and taxonomic composition of the STOCS mycota; 2) to assess the capacity of indigenous fungi to degrade crude oil and; 3) to determine the possible effects of crude oil on the fungal community. Since marine mycology is still in its infancy and no data on the fungi of the western Gulf of Mexico were available, a major effort was directed early in this pilot year toward developing an appropriate methodology. The results of our studies, when integrated with those of other projects will provide information for predicting and measuring the effects of oil and gas development activities upon the STOCS ecosystem.

The significance of marine fungi (those fungi that can survive and presumably grow in the sea) has been frequently overlooked. They are ubiquitous in the marine environment and are important both as decomposers and as parasites. The availability of nitrogen and phosphorous, a limiting factor in the productivity of the continental shelf, is controlled, at least in part, by fungal activity. Fungi serve directly as food for marine invertebrates. A negative impact on the food chain, however, can result from mycoparasitism of diatoms and copepods. Fungal diseases of economic importance also occur among oysters and teleost fish. Clearly, precipitous changes in the fungal community could have far reaching ecological effects.

Perhaps the most significant aspect of marine mycology for BLM is the ability of fungi to penetrate, emulsify and degrade masses of petroleum. It has recently been recognized that fungi are more efficient than bacteria in utilizing petroleum hydrocarbons, but little is known about their capacity to disperse and oxidize all components under natural conditions because most previous studies have incorporated unnaturally high concentrations of nitrogen and phosphorus. An attempt was made to approximate existing nutrient conditions in the studies of the interaction of STOCS fungi and crude oil.

The Marine Mycota

Taxonomy

There have been few previous surveys of the mycota of sublittoral areas (Colwell *et al.*, 1976; Roth *et al.*, 1964; Steele, 1967). Marine mycology has focused almost exclusively on yeasts (Fell, 1967) and on wood-inhabiting filamentous ascomycetes (Barghoorn, 1942; Jones, 1976). Perhaps because Sparrow's pioneering work on marine sediments (Sparrow, 1937) revealed a fungal flora strikingly similar to that of terrestrial soils and air spora, marine microbiologists have questioned the importance and even the existence of free-living saprophytic fungi in the seas (Hughes, 1975). Johnson and Sparrow (1961) isolated fungal filaments from marine mud and obtained from them cultures of *Cephalosporium, Aspergillus, Penicillium, Alternaria, Heterosporium* and *Cladosporium* species, the same "terrestrial" imperfect genera found earlier by Sparrow. Mycelial fragments have been frequently encountered in marine sediments at water depths as great as 4000 m (Morita and Zobell, 1955). That these fungi can grow and colonize new substrates in estuarial waters was demonstrated by the isolation of 30 fungi representing 22 genera from submerged wood panels (Siepmann and Johnson, 1960). All but one belonged to "terrestrial" genera of the Fungi Imperfecti. In cases where marine and estuarial yeasts and free-living filamentous fungi have been identified to species, they were generally found to be species common on land (Ahearn *et al.*, 1962; Borut and Johnson, 1962; Roth *et al.*, 1964; Steele, 1967). It has long been established that yeasts and filamentous soil fungi can tolerate a wide range of salt tolerance (Borut and Johnson, 1962; Gray *et al.*, 1963; Norkrans, 1966).

In addition to the ubiquitous terrestrial species of yeasts and Fungi Imperfecti the seas are also inhabited by a relatively small number of exclusively marine fungi. About 300 such species have been described to date (Jones, 1976). These include members of the primitive zoosporic family, Thraustochytridiaceae (Bahnweg and Sparrow, 1974), and advanced specialized ascomycetes and their imperfect states which are confined to submerged plant material. The fungi found in the areas are precisely those which are able to grow over a wide range of salinities, whereas the growth of those fungal groups not generally found in the seas, the terrestrial ascomycetes, mucoraceous fungi and freshwater hyphomycetes, is inhabited by low concentrations of seawater (Fell *et al.*, 1960; Steele, 1967; Combs *et al.*, 1971; Byrne and Jones, 1975).

Though the growth and reproduction of soil fungi is not inhibited by seawater, spore germination is decidedly reduced by volatile, heat-labile factors which appear to be almost universal in fresh seawater (Borut and Johnson, 1962; Byrne and Jones, 1975; Conrad *et al.*, 1976). Borut and Johnson (1962) found that these inhibitory effects could be reversed by supplementation with carbohydrate or amino acids. They drew attention to the similarity of the marine phenomenon and the universal soil phenomenon of fungistasis. Fungistasis is considered to be due to a balance of inhibition by volatile, heat-labile factors and stimulation by nutrients (Watson and Ford, 1972). This phenomenon has been largely ignored by marine microbiologists even though there appears to be a similar inhibition of bacteria by components in seawater (Dawe and Penrose, 1978). These inhibitory substances might be of microbial origin (Buck *et al.*, 1963). The erratic fungal enumerations reported in the literature might be related to this complex phenomenon.

Abundance

The difficulties encountered in using standard plate count methods for marine fungi have led many authors to report their results as percentage of samples that yield fungi, but some conclusions can be drawn from available enumeration data. Up to 7.5×10^6 colony-forming units (CFU) of filamentous fungi have been obtained from polluted estuarial sediment; the overlying water yielded only 7×10^4 CFU/ℓ respectively (Walker and Colwell, 1974a). The high and low concentrations of yeasts and filamentous fungi in pelagic waters may be related to the productivity of the area as illustrated by the following: North Sea, yeasts < 10/ℓ to 3×10^3/ℓ seawater (Meyers, Ahearn, Gunkel and Roth, 1967); Black Sea, yeasts 0/ℓ to 150/ℓ (Meyers, Ahearn and Roth, 1967); Florida Straits, filamentous fungi 0/ℓ to 50/ℓ; Gulf of Mexico, yeasts 0/ℓ to 80/ℓ (Ahearn *et al.*, 1968); and Indian Ocean, yeasts 0/ℓ to 513/ℓ fungi 0/ℓ to 26/ℓ (Fell, 1967). Van Uden and Castelo-Branco (1963) found that yeasts densities along the Pacific Coast of the United States varied from zero CFU/ℓ to 1.9×10^3/ℓ, whereas along the Atlantic coast yeast numbers varied from < 1/ℓ to 6×10^6/ℓ and filamentous fungi numbered from < 1/ℓ to 6.5×10^5/ℓ (Colwell *et al.*, 1976). Total numbers of fungi in the waters around Mid-Pacific Islands varied from 60/ℓ to 3940/ℓ with an average of 140 CFU/ℓ of water (Steele, 1967). Most of the aforementioned investigators detected a general trend toward higher numbers in the vicinity of land masses, especially in the case of molds. This tendency was found in one study to correlate with nutrient gradients. Threshold concentrations of carbon sources may limit fungal growth in seawater as they do bacterial growth (Jannash, 1970). Marine yeasts were found to increase concurrently with the decline of a *Noctiluca* bloom in the North Sea (Meyers, Ahearn and Roth, 1967) and increase from 80/ℓ to 6800/ℓ of water near a spewing oil well located in the Gulf of Mexico (Ahearn *et al.*, 1971).

Origin

The fact that molds are more restricted than yeasts to the vicinity of land might be, in part, a function of their continual reintroduction from terrestrial sources, either by air transport or freshwater outwash. Pady and Kapica (1955) reported that on the same flight the density of airborne fungal spores varied from 529 spores/ft^3 to 0.5/ft^3. Apparently the spore concentration was a characteristic of air masses and was not related to distance from land, although spore viability decreased with time in the air.

The generic composition of the Atlantic air spora was reported to be the same as that over North Atlantic agricultural lands with spores of *Cladosporium* predominating. Strong evidence against the outwash origin of marine molds came from a comparison of fungal population densities in sediments on the leeward and windward sides of an island. The leeward sediment yielded 3.18 CFU/g, but the windward sediment contained only 0.28 CFU/g even though it was in an area receiving heavy terrestrial outwash (Steele, 1967).

Nutrition

Most marine fungi, including many of the yeasts, have the ability to form filaments which penetrate solid substrates. Extracellular hydrolytic enzymes are secreted by fungi resulting in the release of biologically available forms of nitrogen and phosphorous. In addition to this important role in nutrient cycling, fungi contribute directly to the energy flow by serving as food for grazing invertebrates such as amoebae, flagellates, *Daphnia* and other micro-invertebrates (Cook *et al.*, 1973) and even animals as large as the fiddler crab (Pitts and Cowley, 1974).

Parasitism

Sparrow (1936) was the first to note the impact of a fungal epiphytotic in a marine primary producer when he reported an 88% infection rate by *Ectrogella perforans* in a population of the diatom genus *Licmophora*. Members of the brown algal genus *Fucus* are reportedly severely rotted by a *Cephalosporium* species (Andrews, 1977). Higher up the food chain, copepods are attacked by various fungi (Alderman, 1976; Seki and Fulton, 1969). Atkins (1954) studied a fungus which was able to infect the pea crab as well as two species of barnacle. Another fungal pathogen has been found, however, that is apparently specific to barnacles and can cause up to 100% mortality in egg masses (Johnson, 1958). *Spirolpidium zoophthorum* is potentially economically beneficial as one of the few biological control agents of the common oyster drill (Ganaros, 1957). Other fungi occasionally have damaging economic effects. In the Gulf and Atlantic states the most serious disease of oysters is caused by *Labyrinthomyxa marina* (Ray and Chandler, 1955). Another fungus attacks the larvae of both oysters and clams (Davis *et al.*, 1954). Periodic epizootics in teleost fish have been attributed to fungal parasites (Sproston, 1944; Alderman, 1976).

Petroleum Degradation by Fungi

According to best conservative estimates approximately six million metric tons of petroleum and petroleum products are introduced into estuaries and oceans each year (NAS Workshop, 1975). The hydrocarbonoclastic ability of a wide range of fungi has long been known but only recently has their importance in the biodegradation of oil been recognized. After comparative studies Perry and Cerniglia (1973), Colwell *et al.* (1978 Arch. Microbiol., In Press) and Jones and Edington (1968) concluded that fungi can degrade petroleum more extensively and more efficiently than can bacteria.

The relative recalcitrance to degradation of various classes of hydrocarbons by a number of marine isolates has been determined by Perry and Cerniglia (1973). Compounds were increasingly resistant to fungal degradation in the following order: n-alkanes C_{10}-C_{19}, n-alkenes C_{12}-C_{18}, gases C_2-C_4, alkanes C_5-C_9, branched alkenes to C_{12}, alkenes C_3-C_{11}, branched alkenes, aromatics and cycloalkanes.

Cladosporium resinae has been used as a model organism in hydrocarbon studies because it is the most frequent contaminant of jet fuel (Cofone *et al.*, 1973; Cooney and Walker, 1973). Growth of this species on 10% v/v concentrations of intermediate length (C_{10}-C_{14}) straight chain hydrocarbons as sole carbon and energy sources approached yields on glucose. However, growth decreased as chain length decreased or increased. No growth on gaseous hydrocarbons and no growth on paraffin oils containing n-alkanes C_{29}-C_{31} was detected. Growth was slower on alkenes than on the corresponding alkanes. No growth was recorded on branched alkanes, phenol, naphthalene, anthracene or phenanthrene and only slight growth observed on cycloalkanes, benzene and xylene.

None of 76 fungi isolated from polluted water by Turner and Ahearn (1970) grew on the aromatic, cyclic and heterocyclic compounds tested. Eighteen (18) of the 29 filamentous fungi (11 of 14 genera tested) screened by Lowery *et al* (1968) were able to utilize n-alkanes as sole carbon and energy sources. However, only 11 of 66 (16 genera) yeast isolates had that capacity. Most of the latter represented species of *Candida* with both *Rodotorula* and *Debaryomyces* having only one positive strain.

Many fungi surveyed through the years were found to be able to assimilate n-ketones. Markovetz (1968) also found the ability to utilize n-alkanes to be widespread among filamentous fungi. He also noted that fewer isolates could use the corresponding 1-alkenes.

Degradation of hydrocarbons by fungi may be even more common in nature than batch culture tests indicate. This may be so because alkane oxidation is inhibited by its fatty acid products (Bell, 1971) and also the utilization of one hydrocarbon may be facilitated by another either by cooxidation (Turner and Ahearn, 1970; Ahearn *et al.*, 1971) or increasing uptake (Walker and Cooney, 1975).

From the foregoing discussion of substrate selectivity it is obvious that the extent to which a particular fungus can degrade petroleum will depend upon its composition. Perry and Cerniglia (1973b) found that *Cunninghamella elegans*, *Aspergillus versicolor* and *Eupenicillium* species could degrade 80-90% of a light paraffin-based crude oil in seven days, while degrading only 40-45% of a heavy asphalt-based oil. Conversely, mixed hydrocarbon substrates will support the growth of a limited range of organisms. For example, when 498 strains of yeast belonging to 26 genera were tested for utilization of kerosene as a sole source of carbon and energy, only 58 were positive and of these, 56 were *Candida* species (Komogata *et al.*, 1964). Ahearn *et al.* (1971) discovered such selection occurs in nature among strains of the same species. He found that strains from an oil polluted area grew better on kerosene than those from unpolluted areas. Culturing several strains together on a complex hydrocarbon substrate can increase the percent of substrate degraded. Some microorganisms can utilize n-alkanes while oxidizing cycloparaffins to forms still unavailable to them but available to other microorganisms (Beam and Perry, 1974a). In another study certain combinations of yeasts grew on hydrocarbon substrates which failed to support the growth of individual strains separately (Ahearn *et al.*, 1971). Walker (Walker and Colwell, 1974a) found that yeasts and filamentous fungi in mixed culture degraded up to twice the amount of complex hydrocarbon substrate that they did individually.

Limiting Factors for Fungal Degradation of Oil in Natural Systems

That microbial degradation occurs continuously in nature is evidenced by the composition of residual petroleum in the oceans. This particular oil consists largely of aromatics, isoparaffins and cycloparaffins (Ledet and Laseter, 1974; Brown and Huffman, 1976). These are the petroleum fractions most resistant to microbial oxidation. Under some conditions degradation may be slowed. Blumer *et al.* (1970) found almost no change in the composition of a fuel after two months in estuarial sediment and even after two years some of the readily degradable n-alkanes persisted along with the more recalcitrant iso- and cycloalkanes and aromatic hydrocarbons (Blumer and Sass, 1972). Below a depth of 2.5 cm there was little evidence of even n-alkane removal, suggesting that oxygen could be a limiting factor. Recently oxygen was proven experimentally to limit oil degradation in sediment columns when fine textured material was used (Gibbs and Davis, 1976). It is doubtful, however, that oxygen is limiting in open waters or surficial sediments except where tarballs or coagulated oil masses are concerned.

Temperature is unquestionably a limiting factor in some areas (Atlas *et al.*, 1978) and there is some indirect evidence that it may be more important for fungi than for bacteria (Walker and Colwell, 1974a). Atlas and Bartha (1972a) reported the growth-lag period in crude-oil amended liquid culture was a linear inverse function of the incubation temperature.

Since the lag period was eliminated by using weathered oil, the low temperature effect was attributed to the increased solubility of toxic volatile compounds.

The hydrostatic pressure at extreme ocean depths has been found to limit the rate, but not the extent, of oil degradation by bacteria (Schwarz *et al.*, 1974). The only pertinent information on fungi suggests that pressure on the continental shelf should not be a factor of concern (Morita, 1965).

The lack of hydrocarbonoclastic inoculum does not appear to be a problem, since under non-nutrient limited conditions sediment and water samples from nonpolluted areas, even from abyssal depths, show uniform and significant oil-degrading activity (Conrad *et al.*, 1976; Atlas and Bartha, 1973). However, samples from oil-polluted estuarial waters and sediments exhibit superior activity, especially toward alicyclic and aromatic hydrocarbons, when compared to samples from clean areas (Walker and Colwell, 1974a; Walker and Colwell, 1975).

The availability of nutrients, especially nitrogen and phosphorous, but also iron, is the major factor limiting oil degradation in the marine environment (Dibble and Bartha, 1976; Gibbs and Davis, 1976). Atlas and Bartha (1972b) obtained an eight-fold increase in biodegradation of Sweden crude when they added optimum concentrations of nitrogen (10 mM) and phosphorous (0.35 mM) to natural seawater. Ammonium appears to be a better nitrogen source than NO_3 for crude-oil mineralization (Perry and Cerniglia, 1973a).

Interactions of Fungi and Bacteria

Walker and Colwell (1974a) were able to increase hydrocarbon degradation in petroleum-enriched estuarial sediment samples with increasing concentrations of streptomycin. The decrease in bacterial population was accompanied by an increase in yeast growth. In the absence of antibiotic, the yeast reached log growth phase after seven days and the filamentous fungi reached log phase only after 45 days. Zobell and Prokop (1966) noticed that in still cultures of sediment overlaid with crude oil, fungi, after some weeks, permeated the floating oil and caused it to sink. In another study using natural sediment amended with South Louisiana Crude Oil (SLCO), bacterial numbers decreased while fungal numbers were increasing during the fourth week. One week later, however, chitinolytic bacteria increased dramatically with a concommitant precipitous decline in fungi, most of which have chitinaceous cell walls (Walker *et al.*, 1975). There is an obvious need for an holistic approach in the study of microbial degradation.

Effects of Petroleum on Fungi

Generally, the growth of fungi is eventually stimulated by the presence of petroleum in both artificial culture and in natural situations. Turner and Ahearn (1970) measured an increase in yeast cell density from 30 to 200/ml to 10^4 and 10^5/ml at various stations along a stream that had received a 10,000 gallon waste oil spill. In the Gulf of Mexico, the numbers of yeasts in surface waters jumped from a maximum of 8/100 ml to 1088/100 ml with the intrusion of crude oil from a spewing well (Ahearn *et al.*, 1971). In closely monitored laboratory experiments, an initial decrease in fungal population has been observed upon application of petroleum. This lag in growth activity might be due to the facts that n-alkane-oxidizing enzymes are inductive not constituitive (Cofone *et al.*, 1973) and that one effect of oils on eukaryotes is the inhibition of nucleic acid synthesis and DNA polymerization (Davavin *et al.*, 1975).

The toxicity of an oil to fungi can be a function of its concentration (Turner and Ahearn, 1970) or its composition (Walker and Colwell, 1975a; Walker *et al.*, 1976). Abiotically weathered oil was found to be more toxic to a marine bacterium than the parent material, especially under starvation conditions (Griffin and Calder, 1977).

Mycological Pollution Indicators

The abundance of oil-degrading bacteria (Atlas and Bartha, 1973a) and the relative abundance of these organisms (Walker and Colwell, 1975) have been correlated with existing low levels of pollution. No convincing evidence has been published that the same relation holds true for fungi. It has been established that total numbers of hydrocarbon degraders are not a good predictor of the hydrocarbon degradation potential of an area. The presence in coastal waters of certain species of *Candida*, especially *C. tropicalis*, *C. parapsilosis* and *C. lypolytica* is an indication of general pollution, since these species are usually confined to estuaries where there is a high level of carbon compounds (Van Uden, 1967; LePetit *et al.*, 1970). Wilhm and Dorris (1966) found a low species diversity index of benthic macroinvertebrates to be a useful indicator of oil pollution in a fresh water stream and a similar index has been applied successfully to a Texas estuary (Copeland and Bechtel, 1971). Llanos and Kjoller (1976) found that the fungal community in a soil treated with waste oil had a significantly lower diversity index than a comparable non-treated soil. Since a diversity index is a function of all biotic and abiotic factors acting on a community, it would seem a logical choice as a parameter to be monitored in petroleum impact studies.

MATERIALS AND METHODS

A major objective of the 1977 STOCS-Mycology project was the development of standard methods for the collection, enumeration and isolation of free-living saprophytic marine fungi. Though numerous quantitative studies of marine yeasts have been published in the last 15 years (Fell, 1976), there have been few attempts to enumerate the filamentous fungi on the continental shelf. Extensive tests of methods were conducted parallel to the regular data acquisition. These led to some procedural adjustments but generally confirmed the validity of methods established early in the study.

Sampling

A limited number of line stations was selected for study because of the pioneering nature of the investigation and because of the extensive on-board laboratory manipulation required of mycological specimens. To maximize the information available from 18 water column samples, Stations 1/II, 2/II and 3/II were sampled on six monthly cruises in March, April, July, August, November and December. The locations provided data on a typical STOCS transect comprising a shallow inshore station, an intermediate station and an offshore deep-water site. The addition of Stations 3/I, 2/III and 1/IV to the three stations on Transect II for purposes of seasonal benthic sampling permitted a number of internal comparisons. The latter three stations form a long transect in the study area which is approximately perpendicular to Transect II and which passes from the northernmost offshore station to the southernmost inshore station. This selection of sites allowed the study of gradients parallel to shore, as well as inshore-offshore gradients, while supplying duplicate shallow, intermediate and deep benthic samples. Collections made at these six stations during late winter (March), late spring (June) and fall (October) comprised the 18 benthic samples of the study.

On-board Processing

Benthic

The standard on-board procedures for processing of benthic samples are presented schematically in Figure 9.1. Bottom sediment samples were taken with a 0.025 m^3 Smith-McIntyre grab and the surficial flocculent layer was collected with a sterile spatula and a sterile screw-capped jar. Where the flocculent layer was absent the top 5.0 cm of mineral sediment was collected. All subsequent procedures were performed in a sterilizable transfer hood.

For oil rate-degradation studies (oil component analysis and determination of the effect of crude oil on natural mixed fungal cultures), cultures were established by transfer of 20 ml of sediment to 250-ml screw-capped Erlenmeyer flasks. Each flask contained 80 ml sterile artificial seawater (ASW; Rila Marine Mix with antibiotics, 0.2 M nitrate nitrogen, and 0.5 M phosphate phosphorus) and 0.5% (v/v) autoclave-sterilized Block 54 Southern Louisiana Crude oil (SLCO). Similar cultures without SLCO were prepared to serve as controls. The number of replicate, oil-enriched cultures per sample varied from one for the winter cruise to two for the spring cruise and three for the fall cruise. In addition, triplicate cultures containing 0.1% (v/v) SLCO were prepared from the fall samples.

Filter-sterilized antibiotics were used in both the rate-degradation and enumeration media. The antibiotics were employed in final concentrations as follows:

Winter: Penicillin G 50 mg/ℓ
Streptomycin 25 mg/ℓ

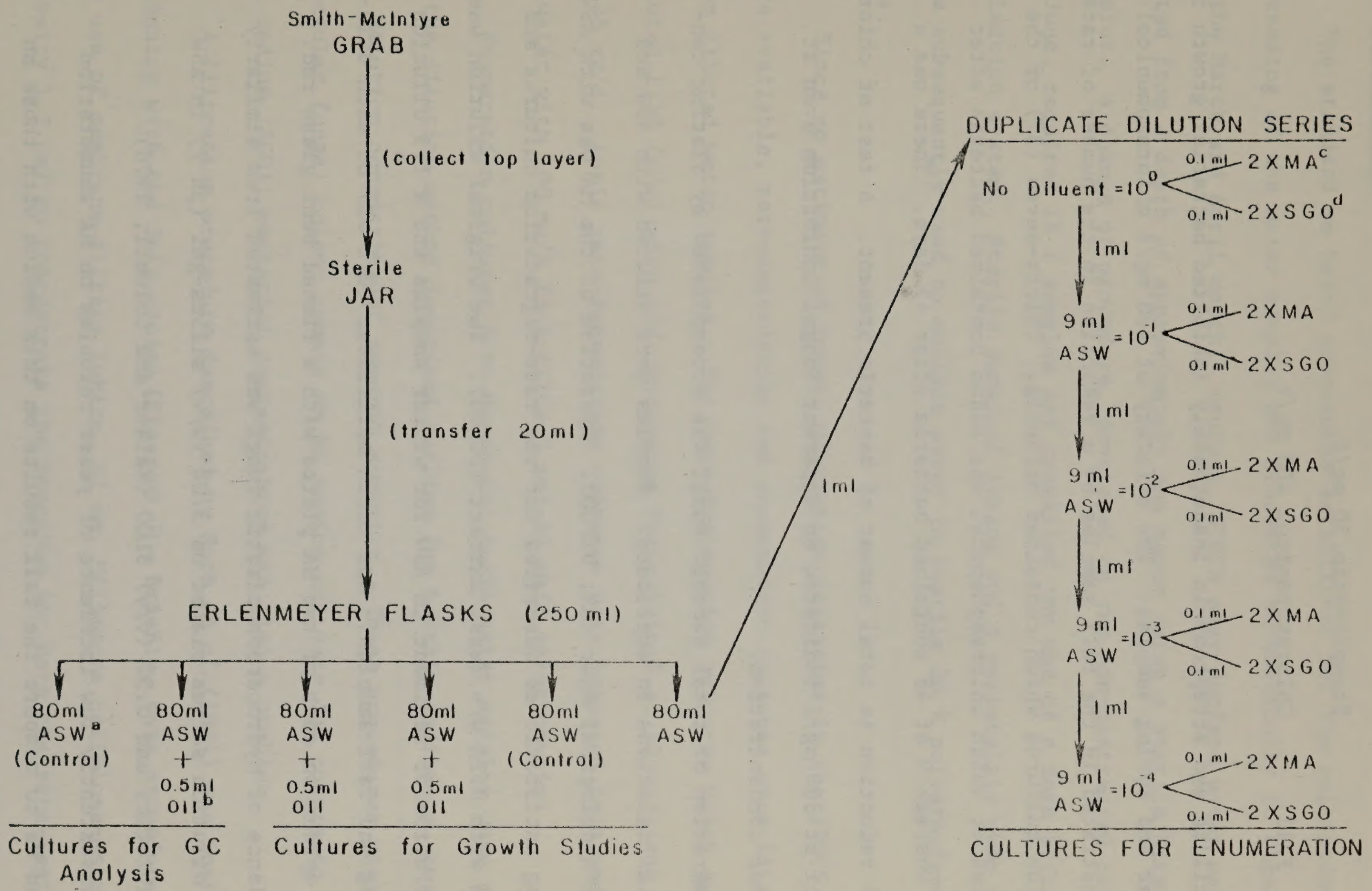

Figure 9.1 Procedure for On Board Processing of Benthic Samples. Artificial Seawater with Antibiotics (a); Southern Louisiana Crude Oil (b); mycological agar plate medium with antibiotics (c); silica-gel oil plate medium with antibiotics (d).

Spring: Penicillin G 100 mg/ℓ

Streptomycin 50 mg/ℓ

Fall: Chloramphenicol 400 mg/ℓ

The penicillin and streptomycin inadequately inhibited bacterial growth in the winter and spring samples. The efficacy of 400 mg/ℓ chloramphenicol was demonstrated in the fall samples by a reduction in the number of rate-degradation cultures which contained bacteria. Thirty-seven (37) of the 48 cultures to which chloramphenical was added contained bacteria after 15 days, whereas 9 of 48 contained bacteria after 45 days. There was a five-fold reduction in total number of bacteria present. A test of chloramphenicol at 800 mg/ℓ indicated no apparent fungal inhibition even at this higher concentration.

Enumeration of total aerobic fungi was accomplished by plating tenfold serial dilutions in duplicate. Samples were diluted with ASW and plated on Mycological Agar (MA; DIFCO). Hydration of the MA was with ASW containing antibiotic as described for the rate-degradation cultures and fortified with 0.1% w/v Yeast Extract (DIFCO). The original dilution was a 1:10 dilution of sediment in ASW for winter samples and a 1:5 dilution for spring and fall samples. One-tenth milliliter (0.1 ml) of each dilution was spread on each of two MA plates with a flamed bent glass rod. The abundance of hydrocarbonoclastic fungi was estimated from similarly prepared dilution series plated on 3.0% (w/v) silica-gel (pH 7) in ASW with antibiotics and 0.5% (v/v) SLCO (Walker and Colwell, 1976).

A consistently low incidence of yeast colonies on MA enumeration plates led us to compare the fall results on this medium with those on Wickerham's Yeast Nitrogen Base medium (DIFCO) plus 1% (w/w) glucose. Yeast colonies appeared only on the MA medium.

Water Column

The standard on board procedures established for the collection and processing of the water samples are presented schematically in Figure 9.2. On the March and April monthly cruises, STOCS near-surface water was collected from a depth of approximately one meter using a sterile Niksin bag sampler. A change of procedure, developed on two non-contracted cruises in June, required 18 ℓ samples and compelled the use of a more efficient collection system. Sterile surgical tubing and a peristaltic pump system were subsequently used to collect water in sterile 20 ℓ polyethylene carboys.

Since no estimate of fungal population densities in the STOCS study area were available, rate-degradation and enumeration cultures were initially made from diluted water samples. For rate-degradation cultures, March samples were diluted 1:10 (v/v) with ASW containing Penicillin G at 50 mg/ℓ and streptomycin at 25 mg/ℓ. April rate-degradation cultures were prepared from undiluted seawater with 100 mg/ℓ Penicillin G and 50 mg/ℓ streptomycin.

Enumeration cultures from March and April water samples were prepared by the dilution method described earlier for benthic sediments, but with the 10° dilution consisting of natural seawater. These procedures yielded irregular fungal colony counts on MA medium and very low counts on silica-gel oil medium (SGO); bacterial contamination was also a problem.

A filtration procedure described by Roth *et al.* (1964) was instituted beginning with the July cruise (Figure 9.2). Briefly, this procedure involved the use of membrane filters (white, 47 mm, Millipore cellulose-ester with porosity 1.2 μm) to concentrate the inoculum for both the rate-degradation and the enumeration cultures. After filtering 900 ml of sample water through each membrane, natural-mixed cultures were established by placing the filters

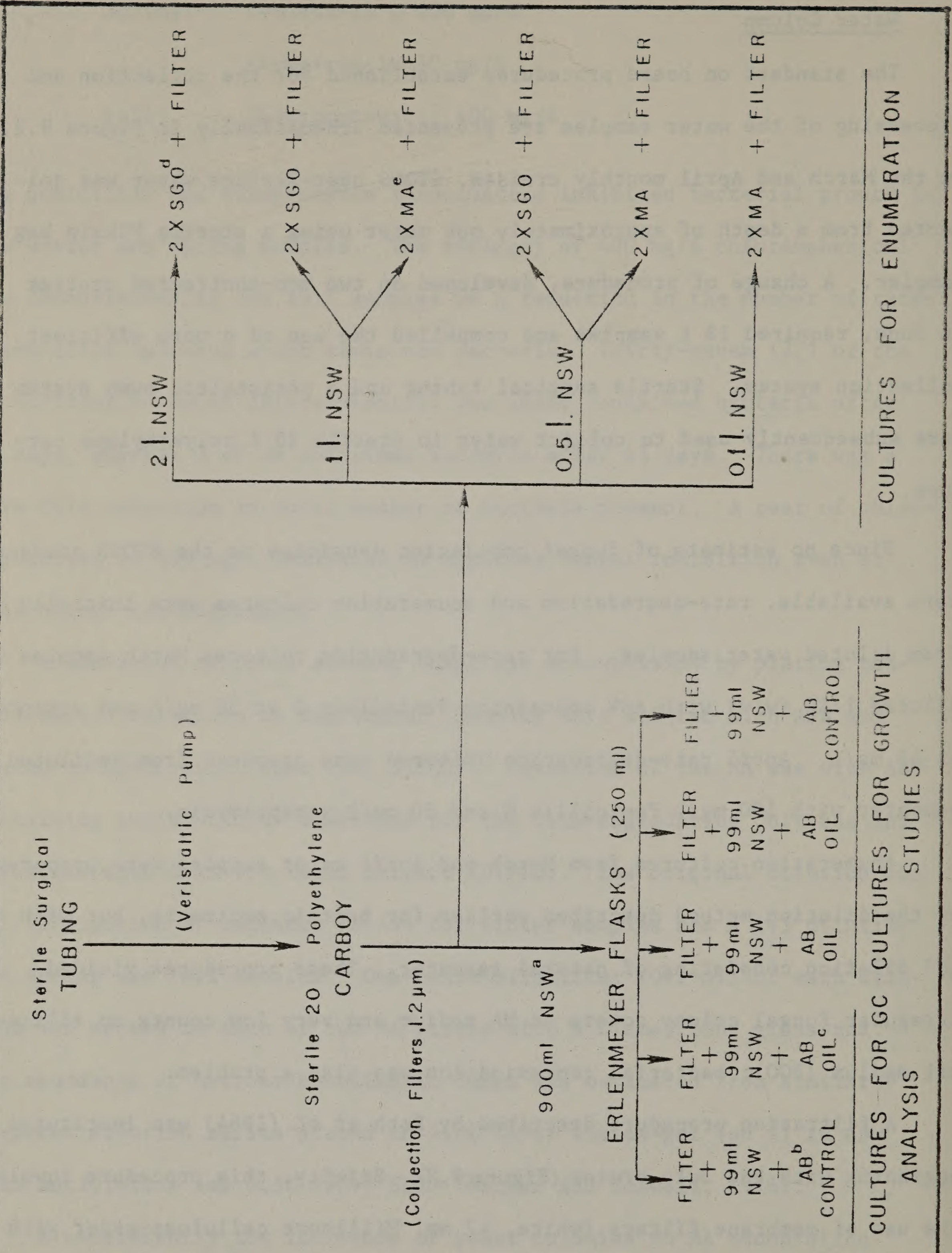

Figure 9.2 Procedure for On Board Processing of Water Samples. Natural Seawater (a); Antibiotics (b); Southern Louisiana Crude Oil at a Final Concentration of 0.5% v/v (c); Silica-Gel Oil Plate Medium with Antibiotic (d); and Mycological Agar Plates with Antibiotics (e).

in 250-ml Erlenmeyer screw-capped flasks containing 99 ml water sample with or without 0.5% SLCO and filter-sterilized antibiotic (400 mg/ℓ chloramphenicol). This procedure effected a ten-fold concentration of the inoculum. In order to study the effect of different concentrations of oil on natural mixed fungal cultures, July and August samples were also enriched with 0.1% SLCO for comparison with the standard 0.5% concentration.

Enumeration of total aerobic fungi was accomplished by placing filters directly on duplicate MA plate medium after filtration of 1.0, 0.5 and 0.1 ℓ of each sample. Similarly, duplicate SGO plates for the enumeration of hydrocarbonoclastic fungi, received filters after filtration of 2.0, 1.0 and 0.5 ℓ of sample. Chloramphenicol at 400 mg/ℓ was the antibiotic used in enumeration media in July and thereafter.

Since colony counts on SGO of hydrocarbonoclastic fungi had been very low with March and April samples, samples from July, November and December were also plated on silica-gel medium with 0.5% (v/v) n-hexadecane. The hexadecane was used as a control to evaluate the possibility of inhibition of fungal growth by the crude oil. Subsequent comparisons indicated that there were, in fact, no significant differences in the enumeration of hydrocarbonoclastic fungi in the presence of SLCO or n-hexadecane. The frequency of yeast colonies detected in the August water samples was not improved in Yeast Nitrogen Base medium compared to the standard MA medium.

The sterility of the transfer hood was monitored at each station on both benthic and water-column cruises by exposing sterile control media for approximately 45 seconds. Only 4 of the 36 controls were positive during the year. Two of the six controls from the March cruise, one with three fungal colonies and another bearing a single colony, indicated a potential contamination problem. Measures were taken to insure the sterility of the work area.

Laboratory Processing

Enumeration Cultures

All enumeration cultures were incubated at 25°C. The MA cultures were examined microscopically and isolations performed after 3, 7 and 14 days. The SGO cultures were similarly examined after 3, 14 and 30 days. At these times, the number of colonies were enumerated and each fungal type described according to colonial morphology. Slope cultures of each type were established on MA medium. Slope cultures showing identical cultural characteristics were grouped, given a code number, and a typical representative of each group was entered into the permanent culture collection. This procedure proved to be very conservative. It is doubtful that there were any species present which were not detected and isolated. The permanent culture collection included several isolates of most common species.

For the purpose of identification, Riddell slide cultures were prepared (Riddell, 1950) of all viable isolates in the culture collection. Each slide culture yielded two permanent slides. All sporulating filamentous isolates were identified to genus and many to species. Identification was accomplished using the standard taxonomic literature based on gross colonial and microscopic characteristics. Yeast isolates were identified to genus and in a few cases to species by a combination of physiological and morphological characters. A synoptic key of these characters was prepared especially for the identification of the yeast isolates of the STOCS project.

Silica-Gel, Oil, Tube-Assay

Assimilation of hydrocarbons by STOCS fungal isolates was measured semi-quantitatively using a silica-gel, oil, tube-assay (Nyns *et al.*, 1968). Each isolate was inoculated onto duplicate slopes of Wickerman's Yeast Nitrogen

Base medium rehydrated with ASW and solidified with 3% Silica-gel (final pH 5.0). The only available carbon and energy source in this medium was glucose (0.01%) which was added to establish the culture. After incubation at 25°C for one week, SLCO was added to one culture of each isolate so as to submerge one-half of the culture. The isolates were scored after one month for growth (*e.g.* good growth, weak growth and no growth) above and below the oil surface. The amount of growth was determined by comparing the amount of mycelium present in the oil-enriched and the control cultures.

Rate-Degradation Cultures

Rate-degradation cultures were processed according to the procedures outlined as follows. Cultures were incubated at 25°C on a gyratory shaker (New Brunswick Model G-10) rotating at 250 rpm. In addition to the cultures prepared on board, abiotic-weathering control flasks containing 0.5% (v/v) autoclaved-SLCO in 100 ml of ASW were similarly incubated. The effects of crude oil on fungal population densities and succession were assessed by the dilution plate method. A 2.0 ml aliquot was removed from each flask and diluted with ASW in a four step 1:10 (v/v) series; 0.1 ml of each dilution was plated in duplicate on MA medium. Samples for dilution plating were removed at 10-day intervals from both oil-enriched and control cultures. Fifteen (15)-day intervals were used for the fall benthic samples and for November and December water samples. MA dilution plates were incubated for one week at 25°C. Fungal colonies arising on these plates were enumerated, isolated and processed as previously described for those colonies originating in enumeration cultures.

Incubation of rate-degradation cultures was terminated after 20, 40 and 45 days for the winter, spring and fall benthic collections, respectively. Rate-degradation cultures established using water column samples were incubated for 20 days (March), 50 days (April), 40 days (July and August) and 45 days (November and December). Upon termination of incubation, the benthic-derived, rate-degradation, cultures and control cultures were frozen and transmitted to Dr. P.L. Parker for extraction and analysis of hydrocarbons. Rate-degradation cultures derived from water column samples and the controls were processed immediately after termination of incubation. The contents of each flask were extracted twice with analytical grade benzene. The benzene extract was evaporated to a volume of 1.5 ml and stored in a tube sealed with a teflon membrane. Standard methods used for sediment extraction and quantitative hydrocarbon analyses are described in the section of this report dealing with high molecular weight hydrocarbons in STOCS sediments.

RESULTS

STOCS Mycota

Water Column

The average fungal population density for the year was a rather low 0.01 colony-forming units (CFU) per ml in water column samples. The highest concentration, 84 CFU/ml, was recorded during March. Populations of this magnitude were detected at all water column stations (1/II, 2/II and 3/II) on both the March and April cruises (Figure 9.3; Appendix H, Table 1). Fungal abundance in July, August, November and December varied between 0.001 and 0.7 CFU/ml. In November, a slight secondary peak of questionable significance was detectable. The lowest monthly average was that of August with 0.004 CFU/ml. A decrease in abundance toward shore was evident in the July samples but over the year the intermediate station yielded significantly fewer fungi while the total number collected at the other stations was identical.

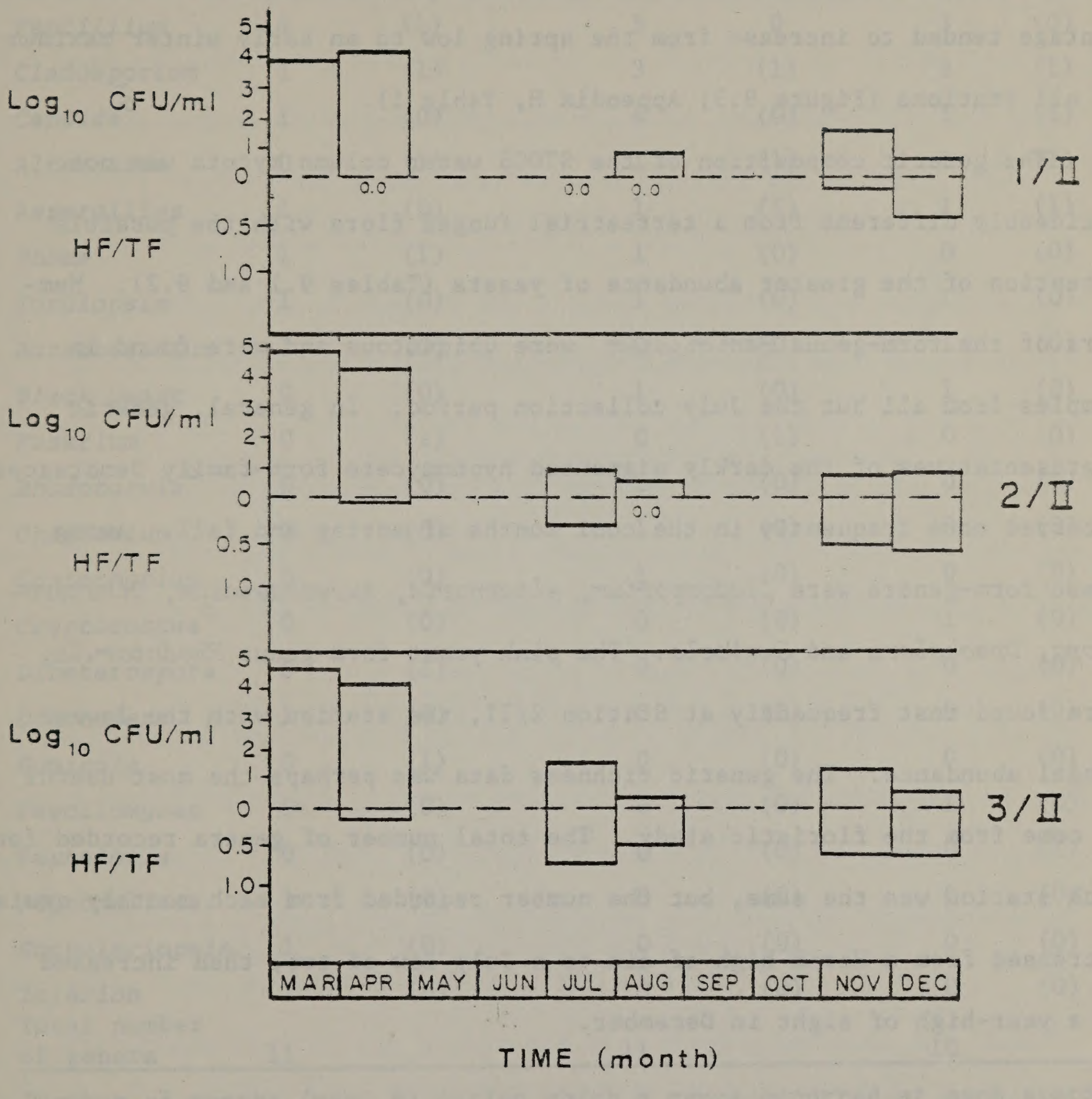

Figure 9.3 Fungal Population Densities ($\log_{10}$ CFU/ℓ of seawater) and the Ratio of Hydrocarbonoclastic Fungi to Total Fungi (HF/TF) in STOCS Near-Surface Water. Enumerations of Hydrocarbonoclastic Fungi were Performed on Hydorcarbonoclastic Medium. Enumerations of Total Fungi were Performed on Mycological Agar Medium.

The percentage of fungi that were able to degrade crude oil (SLCO) ranged from a low of 0% at the inshore station during the spring and summer months to a high of 70 to 67% in the November and December samples from Station 3/II. A very consistent offshore gradient of increasing relative abundance of oil degraders was detected throughout the year and the percentage tended to increase from the spring low to an early winter maximum at all stations (Figure 9.3; Appendix H, Table 1).

The generic composition of the STOCS water column mycota was not noticeably different from a terrestrial fungal flora with the possible exception of the greater abundance of yeasts (Tables 9.1 and 9.2). Members of the form-genus *Penicillium* were ubiquitous and were found in samples from all but the July collection period. In general, generic representatives of the darkly pigmented hyphomycete form-family Demateaceae occurred more frequently in the cool months of spring and fall. Among these form-genera were *Cladosporium*, *Alternaria*, *Aureobasidium*, *Diheterospora*; *Drechslera* and *Humicola*. The pink yeast form-genus *Rhodotorula*, were found most frequently at Station 2/II, the station with the lowest fungal abundance. The generic richness data was perhaps the most useful to come from the floristic study. The total number of genera recorded for each station was the same, but the number recorded from each monthly cruise decreased from a March high of six to a July low of two, then increased to a year-high of eight in December.

Benthic

The year's average fungal population density in benthic samples was 236 CFU/ml sediment. The greatest concentration, 1600 CFU/ml, was recorded in the fall sample from Station 3/II. There was a general progression toward larger fungal populations from the late-winter low to the fall high

TABLE 9.1

OCCURRENCES[1] (BY STATION) OF FUNGAL GENERA IN WATER COLUMN SAMPLES

Genus	Station					
	1/II		2/II		3/II	
	MA[a]	SGO[b]	MA[a]	SGO[b]	MA[a]	SGO[b]
Pencillium	4	(1)	5	0	3	(0)
Cladosporium	1	(1)	3	(1)	2	(1)
Candida[c]	1	(0)	4	(0)	1	(1)
Alternaria	0	(1)	1	(1)	1	(1)
Aspergillus	1	(0)	1	(1)	1	(1)
Phoma	1	(1)	1	(0)	0	(0)
Torulopsis	1	(0)	1	(0)	1	(0)
Aureobasidium	1	(0)	0	(0)	1	(0)
Black yeast	0	(0)	1	(0)	1	(0)
Fusarium	0	(1)	0	(1)	0	(0)
Rhodotorula[c]	0	(0)	2	(0)	0	(0)
Chaetomium	0	(0)	1	(0)	0	(0)
Coniothyrium	0	(0)	1	(0)	0	(0)
Cryptococcus[c]	0	(0)	0	(0)	1	(0)
Diheterospora	0	(1)	0	(0)	0	(0)
Drechslera	1	(0)	0	(0)	0	(0)
Humicola	0	(1)	0	(0)	0	(0)
Paecilomyces	0	(0)	0	(0)	1	(0)
Pestalotia	0	(0)	0	(0)	0	(1)
Peyronellaea	1	(0)	0	(0)	0	(0)
Scopulariopsis	1	(0)	0	(0)	0	(0)
Zalerion	1	(0)	0	(0)	0	(0)
Total number of genera	11		11		10	

[1] Number of months (max. 6) during which a genus occurred at each station.

[a] Occurrence on Mycological Agar plates (non-selective)

[b] Occurrence on Silica-gel Oil plates (selective for hydrocarbonoclastic fungi).

[c] Yeast.

TABLE 9.2

OCCURRENCE[1] (BY MONTH) OF FUNGAL GENERA IN WATER COLUMN SAMPLES

Genus	Month											
	March		April		July		August		November		December	
	MA[a]	SGO[b]	MA[a]	SGO[b]	MA[a]	SGO[b]	MA[a]	SGO[b]	MA[a]	SGO[b]	MA[a]	SGO[b]
Penicillium	2	(0)	3	(0)	0	(0)	3	(0)	2	(0)	2	(1)
Cladosporium	2	(0)	0	(0)	0	(0)	1	(0)	3	(0)	0	(3)
Candida[c]	1	(0)	1	(0)	0	(1)	2	(0)	0	(0)	2	(0)
Alternaria	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	2	(3)
Aspergillus	1	(0)	0	(0)	2	(1)	0	(1)	0	(0)	0	(0)
Phoma	0	(0)	0	(0)	0	(0)	0	(0)	2	(1)	0	(0)
Torulopsis[c]	0	(0)	0	(0)	1	(0)	0	(0)	1	(0)	1	(0)
Aureobasidium	0	(0)	2	(0)	0	(0)	0	(0)	0	(0)	0	(0)
Black yeast	0	(0)	2	(0)	0	(0)	0	(0)	0	(0)	0	(0)
Fusarium	0	(0)	0	(0)	0	(0)	0	(0)	0	(2)	0	(0)
Rhodotorula[c]	1	(0)	0	(0)	0	(0)	0	(0)	0	(0)	1	(0)
Chaetomium	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	1	(0)
Coniothyrium	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	1	(0)
Cryptococcus[c]	1	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
Diheterospora	0	(0)	0	(0)	0	(0)	0	(0)	0	(1)	0	(0)
Drechslera	0	(0)	1	(0)	0	(0)	0	(0)	0	(0)	0	(0)
Humicola	0	(0)	0	(0)	0	(0)	0	(0)	0	(1)	0	(0)
Paecilomyces	0	(0)	0	(0)	0	(0)	0	(0)	1	(0)	0	(0)
Pestalotia	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(1)
Peyronellaea	0	(0)	0	(0)	0	(0)	0	(0)	1	(0)	0	(0)
Scopulariopsis	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	1	(0)
Zalerion	0	(0)	0	(0)	0	(0)	0	(0)	1	(0)	0	(0)
Total number of genera	6		5		2		3		7		8	

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[1] Number of stations (max. 3) at which a genus occurred during each monthly cruise.

[a] Occurrence on Mycological Agar plates (non-selective).

[b] Occurrence on Silica-gel Oil plates (selective for hydrocarbonoclastic fungi).

[c] Yeast.

(Figure 9.4; Appendix H, Table 2). No intelligible spatial patterns were discernible, however, there was a strong correlation (r = 0.87) between the annual averages of total sediment organic matter and fungal population density (Figure 9.5). The calculated percentage of fungi capable of degrading oil varied radically in the benthic samples from a low of 12% to a high of 290%; most values exceeded 50% (Appendix H, Table 2). An artifactual excess of hydrocarbonoclastic over total fungi has occurred in previous studies using the same methods (Walker and Colwell, 1974; Colwell *et al.*, 1976).

The generic composition of the benthic mycoflora was very similar to that of the water column. Here too, *Penicillium* spp. were predominant, occurring at all stations in all collection periods (Tables 9.3 and 9.4). The dermatiaceous form-genera were again more abundant in the cool seasons. Generic richness was constant among seasons, but varied greatly between stations, from a low of three at Station 3/I to highs of eight at 3/II and nine at 1/IV. There was no correlation of generic richness with depth. A comparison of generic frequencies in water column and benthic samples showed that fungi which occur as yeasts, including *Aureobasidium* sp., were encountered more frequently in water (Table 9.5). Other genera, notably the form-genera *Aspergillus, Cephalosporium* and *Fusarium* were recovered more often from sediment than from water samples.

A complete list of the genera and form-genera isolated and their origins is presented in Appendix H, Table 3. Of the 43 genera encountered only *Zalerion* is considered to be exclusively marine. There were 16 pigmented and 11 non-pigmented hyphomycete form-genera, but only four form-genera of Coelomycetes and five imperfect yeasts. Sexual fungi were scarcely in evidence with only five ascomycete genera including a yeast and a single basidiomycetous yeast being encountered. A single species of

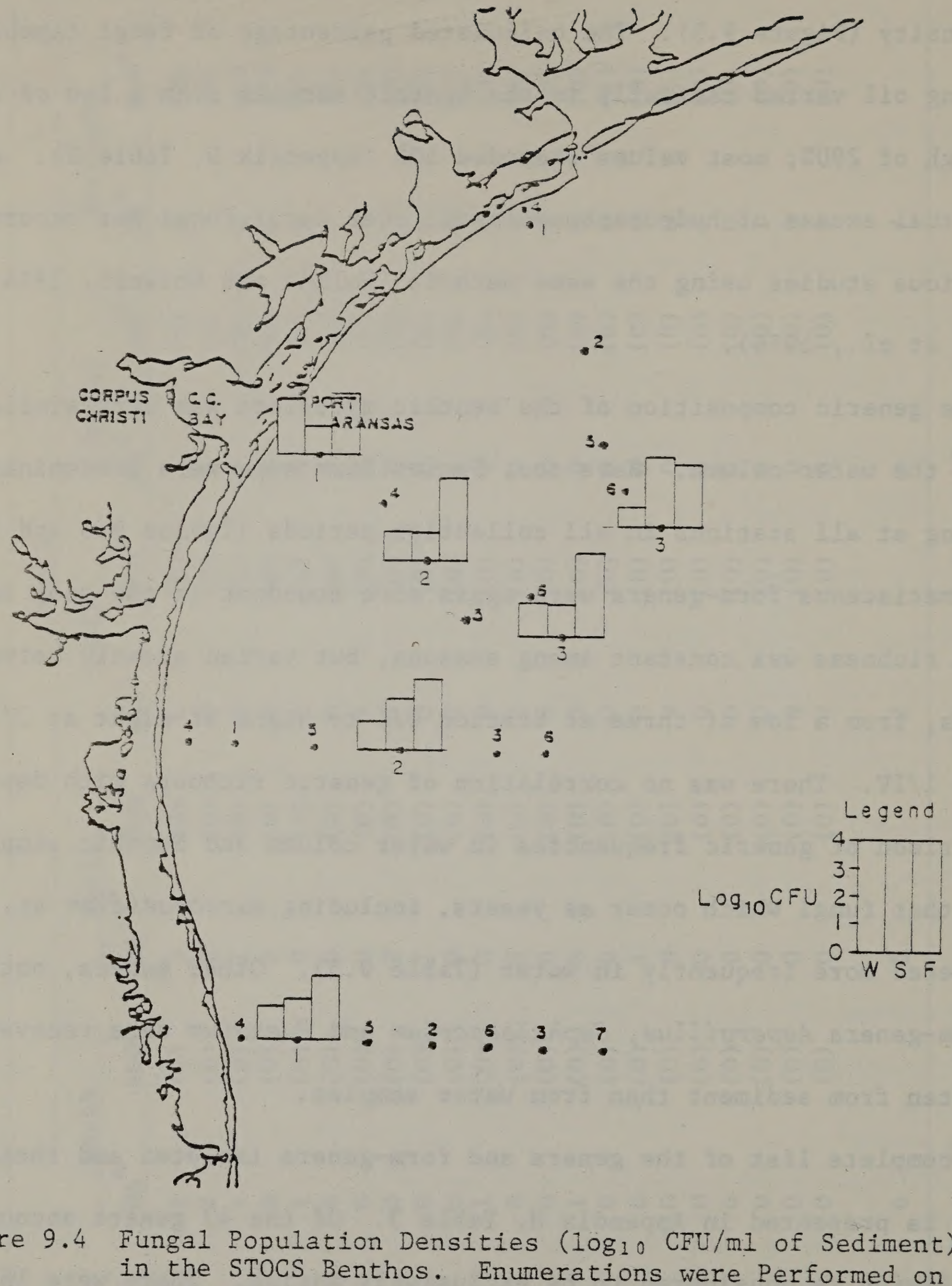

Figure 9.4 Fungal Population Densities (log$_{10}$ CFU/ml of Sediment) in the STOCS Benthos. Enumerations were Performed on Mycological Agar Dilution Plates. Symbols: W = winter, S = spring, F = fall.

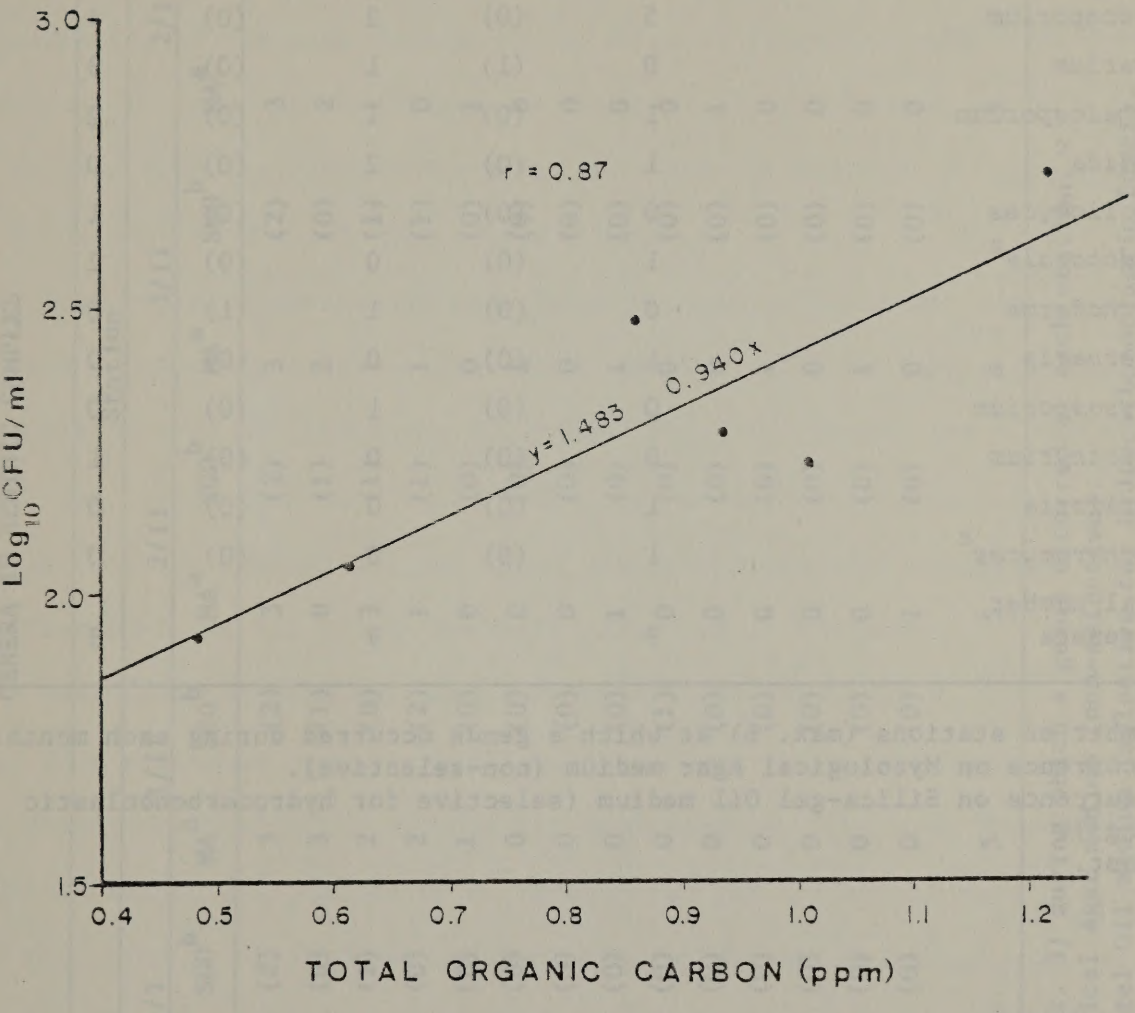

Figure 9.5 Correlation of Fungal Population Density and Total Organic Carbon in STOCS Sediment. The Numbers for Both Fungal Population Density and Total Organic Carbon are Based on Annual Station Averages.

TABLE 9.3

SEASONAL OCCURRENCE[1] OF FUNGAL GENERA IN BENTHIC SAMPLES

Genus	Season					
	Winter		Spring		Fall	
	MA[a]	SGO[b]	MA[a]	SGO[b]	MA[a]	SGO[b]
Penicillium	4	(0)	6	(6)	6	(4)
Aspergillus	3	(0)	3	(3)	5	(1)
Cladosporium	5	(0)	2	(0)	4	(3)
Fusarium	0	(1)	1	(0)	3	(4)
Cephalosporium	1	(0)	1	(0)	2	(1)
Candida[c]	1	(0)	2	(0)	0	(0)
Paecilomyces	0	(0)	1	(0)	1	(1)
Rhodotorula[c]	1	(0)	0	(0)	2	(0)
Trichoderma	0	(0)	1	(1)	0	(0)
Alternaria	1	(0)	0	(0)	0	(0)
Chrysosporium	0	(0)	1	(0)	0	(0)
Coniothyrium	0	(0)	0	(0)	1	(0)
Pestalotia	1	(0)	0	(0)	0	(0)
Saccharomyces[c]	1	(0)	0	(0)	0	(0)
Total number of genera	9		9		8	

[1] Number of stations (max. 6) at which a genus occurred during each month.

[a] Occurrence on Mycological Agar medium (non-selective).

[b] Occurrence on Silica-gel Oil medium (selective for hydrocarbonoclastic fungi).

[c] Yeast.

TABLE 9.4

OCCURRENCE[1] (BY STATION) OF FUNGAL GENERA IN BENTHIC SAMPLES

Genus	Station											
	3/I		1/II		2/II		3/II		2/III		1/IV	
	MA[a]	SGO[b]	MA[a]	SGO[b]	MA[a]	SGO[b]	MA[a]	SGO[b]	MA[a]	SGO[b]	MA[a]	SGO[b]
Penicillium	2	(2)	3	(2)	3	(2)	3	(2)	3	(1)	2	(1)
Aspergillus	2	(0)	3	(1)	0	(1)	2	(0)	2	(2)	2	(0)
Cladosporium	3	(1)	2	(0)	3	(1)	1	(1)	1	(0)	1	(0)
Fusarium	0	(0)	2	(2)	1	(1)	1	(1)	0	(0)	0	(1)
Cephalosporium	0	(0)	1	(0)	0	(0)	0	(0)	1	(0)	2	(1)
Candida[c]	0	(0)	0	(0)	0	(0)	1	(0)	0	(0)	2	(0)
Paecilomyces	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	2	(1)
Rhodotorula[c]	0	(0)	0	(0)	1	(0)	1	(0)	0	(0)	1	(0)
Trichoderma	0	(0)	0	(1)	0	(0)	0	(0)	0	(0)	1	(0)
Alternaria	0	(0)	0	(0)	0	(0)	0	(0)	1	(0)	0	(0)
Chrysosporium	0	(0)	0	(0)	0	(0)	1	(0)	0	(0)	0	(0)
Coniothyrium	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	1	(0)
Pestalotia	0	(0)	0	(0)	0	(0)	1	(0)	0	(0)	0	(0)
Saccharomyces[c]	0	(0)	0	(0)	1	(0)	0	(0)	0	(0)	0	(0)
Total Number of genera	3		5		5		8		5		9	

---

[1] Number of seasons (max. 3) during which a genus occurred at each station.

[a] Occurrence on Mycological Agar medium (non-selective).

[b] Occurrence on Silica-gel Oil medium (selective for hydrocarbonoclastic fungi).

[c] Yeast.

TABLE 9.5

WATER COLUMN/BENTHOS HABITAT PREFERENCE OF FUNGAL GENERA[1]

Genus	Water Column	Benthos
Torulopsis[a]	3	0
Aureobasidium	2	0
Black yeast	2	0
Phoma	2	0
Chaetomium	1	0
Cryptococcus[a]	1	0
Drechslera	1	0
Peyronellaea	1	0
Scopulariopsis	1	0
Zalerion	1	0
Candida[a]	6	3
Alternaria	2	1
Coniothyrium	1	1
Penicillium	12	16
Rhodotorula[a]	2	3
Cladosporium	6	11
Paecilomyces	1	2
Aspergillus	2	11
Chrysosporium	0	1
Pestalotia	0	1
Saccharomyces[a]	0	1
Trichoderma	0	1
Cephalosporium	0	4
Fusarium	0	4

[1] Frequency of occurrence in 18 water column and 18 benthic samples on Mycological Agar medium.

[a] Yeast

zygomycete, *Syncephalastrum racemosum* (Cohn) Schroeter, was isolated.

Species Identification

Many of the fungi isolated in these studies were identified to species. Of these species, most have been reported previously from marine or estuarian habitats. However, most all were considered to be terrestrial. By far the most common *Penicillium* form-species was *P. citrinum* Thom. Other form-species of the same form-genus in order of frequency were *P. piscarium* Westling, *P. cyano-fulvum* Biourge, *P. lanosos-viride* Thom, *P. steckii* Zaleski, *P. rolfsii* Thom, *P. lanosum* Westline, *P. nigricans* Bainier, *P. rubrum* Stoll, *P. spinulosum* Thom and *P. stoloniferum* Thom.

Aspergillus form-species were found primarily in benthic samples and in rate degradation cultures from water samples. The two most frequently encountered were *A. flavus* var *columnaris* Raper and Fennel and *A. sydowii* (Bain and Sart.) Thom and Church. The next most common *Aspergillus* was a strain from the fumigatus group which was morphologically similar to *A. viridi-nutans* but had colony characteristics of *A. paradoxus*.

Some *Fusarium* strains, isolated mainly from the benthos, deviated somewhat from published species descriptions, a fact which suggested evolutionary divergence from their terrestrial counterparts. The most common *Fusarium* strain was similar to *F. ventricosum* and the second most abundant was most like *F. moniliforme* and *subglutenans* Wr. and Reink.

A majority of the isolates of the very abundant form-genus *Cladosporium* belonged to the ubiquitous species *C. cladosporioides* (Fresen.) deVries. *Candida diddensii* (Phaff, Mrak et William) Fell et Meyer, a marine imperfect yeast first described from the STOCS area and another imperfect yeast, *Cryptococcus albidus* Kutzing emerd. Poeffet Spencer, were also identified among STOCS isolates.

Oil Degradation

Natural Mixed Cultures

The ability of the fungi in each sample to degrade the n-alkanes in crude oil was determined by gas-chromatographic analysis. The relative quantities of pristane, phytane and normal alkanes C_{16} and C_{32} remaining after several weeks incubation were determined.

The ratios of pristane/n-hepatadecane, phytane/n-octadecane and the sum of normal alkanes from C_{16} to C_{32}/the sum of pristane and phytane were calculated for water samples (Appendix H, Table 4) and sediment samples (Appendix H, Table 5). Differences in these ratios between samples from the same cruise were assumed to be proportional to differences in the oil-degradation potential of the samples.

Because different incubation times and analyzers were used on the samples from different cruises, the data were normalized to the ratio indicating the greatest oil degradation among the samples from each cruise. The average of the normalized ratios for each sample are presented for intra-cruise comparisons in Figures 9.6 and 9.7. It should be kept in mind that the absolute amount of oil degraded is in no way reflected by these data.

The fungal, oil-degrading potential of water from the inshore station 1/II appeared to be superior to that of the offshore stations during the spring and early summer. However, all differences disappeared by August. Slightly better degradation was accomplished in samples from 2/II than from those of 3/II during the same period.

A similar, but more pronounced, offshore decrease in degradation potential was seen when the benthic stations from Transect II were compared. The effect was most pronounced in June though it was still present in the fall samples. When all six benthic stations were considered together, 1/II,

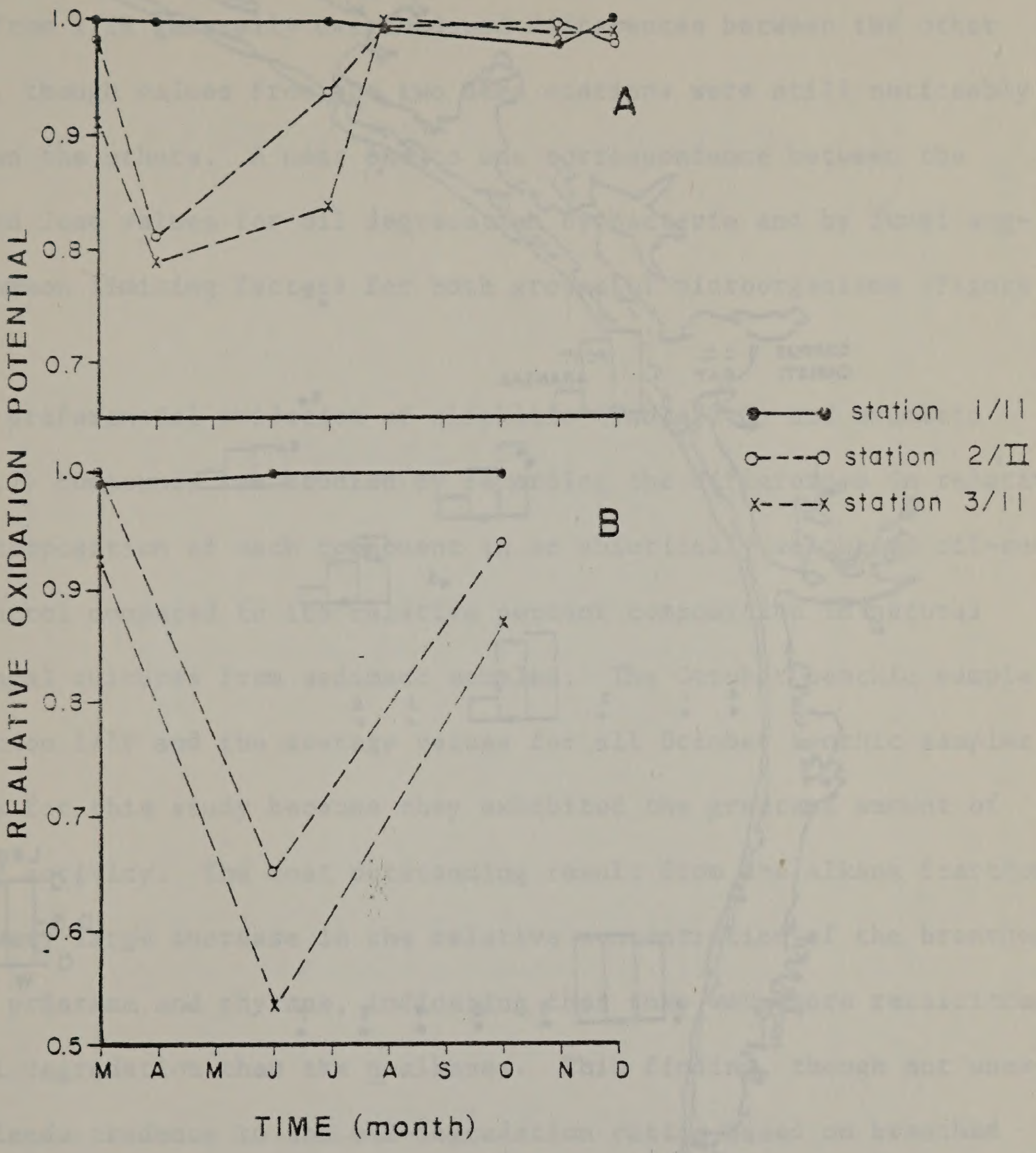

Figure 9.6 Relative n-alkane Oxidation Potential of the STOCS Near-Surface Water Column (A) and Benthic (B) Mycota by Month at Stations 1/II, 2/II and 3/II. The Relative Oxidation Potential was Based on the Station Exhibiting the Greatest n-alkane Degradation at Each Collection Period as Indicated by the Ratios Pristane/n-C_{17}, Phytane/n-C_{18} and Σn-C_{16}-C_{32}/(pristane and phytane).

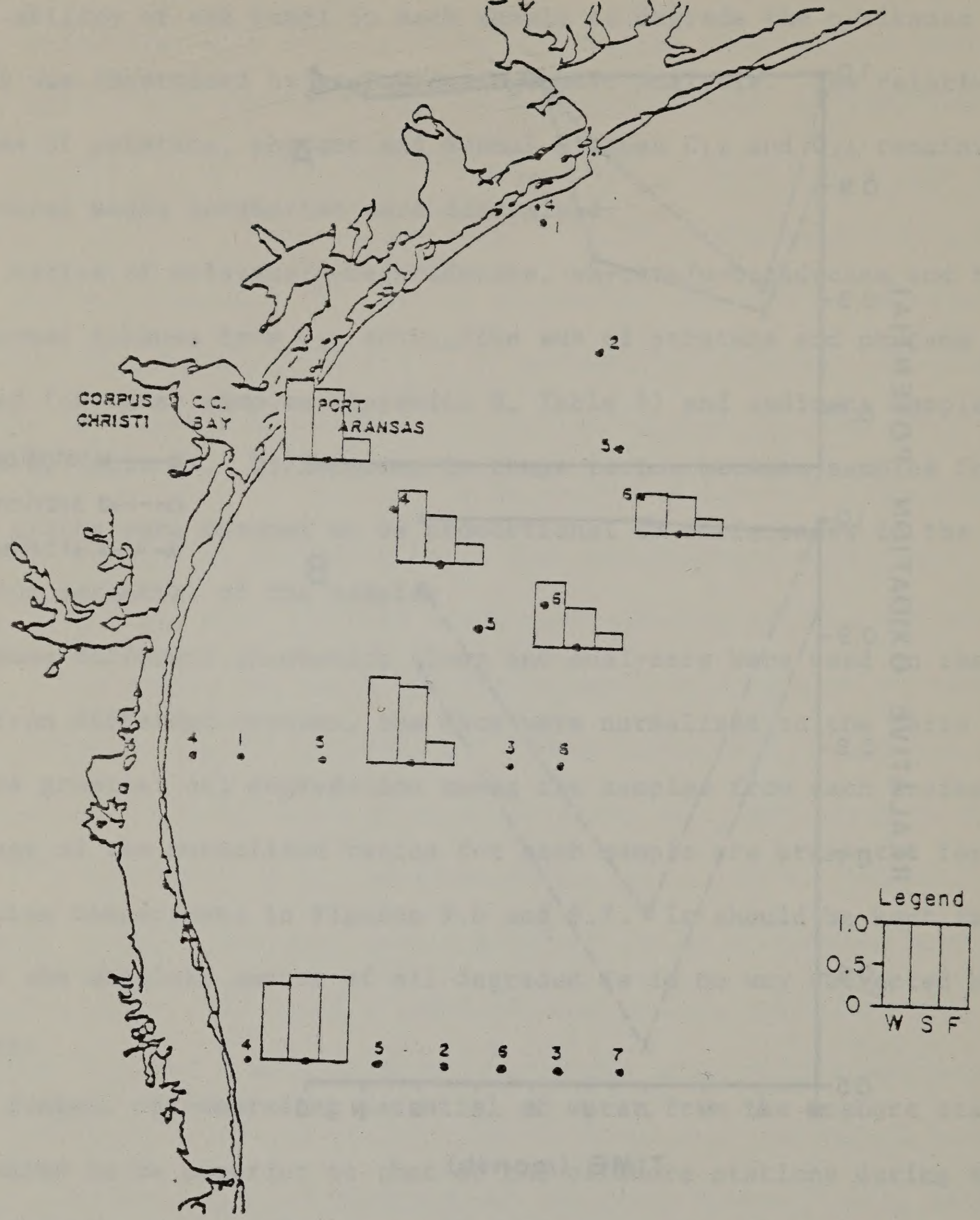

Figure 9.7 Relative n-alkane Oxidation Potential of the STOCS Benthic Mycota by Season. Potentials were Based on the Station Exhibiting the Greatest n-alkane Degradation at Each Collection Period as Indicated by the Ratios Pristane/n-C_{17}, phytane/n-C_{18}, and n-C_{16}-C_{32}/pristane and phytane. W = winter, S = spring, F = fall.

2/III and 1/IV exhibited high relative degradation rates in late winter and late spring, whereas the lowest values occurred at Stations 3/I and 3/II (Figure 9.7). The very strong oil degradation shown by the fall samples from 1/IV generally overshadowed differences between the other stations, though values from the two deep stations were still noticeably lower than the others. A near one to one correspondence between the normalized June values for oil degradation by bacteria and by fungi suggested common limiting factors for both groups of microorganisms (Figure 9.8).

The preferential oxidation of aliphatic (Table 9.6) and aromatic (Table 9.7) compounds was studied by recording the differences in relative percent composition of each component in an abiotically weathered oil-seawater control compared to its relative percent composition in natural mixed fungal cultures from sediment samples. The October benthic sample from Station 1/IV and the average values for all October benthic samples were used for this study because they exhibited the greatest amount of degrading activity. The most outstanding result from the alkane fraction was the very large increase in the relative concentration of the branched alkanes, pristane and phytane, indicating that they were more recalcitrant to fungal degradation than the n-alkanes. This finding, though not unexpected, lends credence to the oil-degradation ratios based on branched chain/unbranched chain ratios. Another, unidentified, compound with a Kovats index of 1851 proved to be resistent to degradation. A clear preference was shown for the utilization of n-alkanes of intermediate chain length C_{16} to C_{22}, especially n-heptadecane and n-octadecane. Longer chain n-alkanes showed a lower but somewhat uneven rate of utilization. There was some evidence that the preference pattern varied with oil concentration. A similar but more pronounced concentration dependence existed

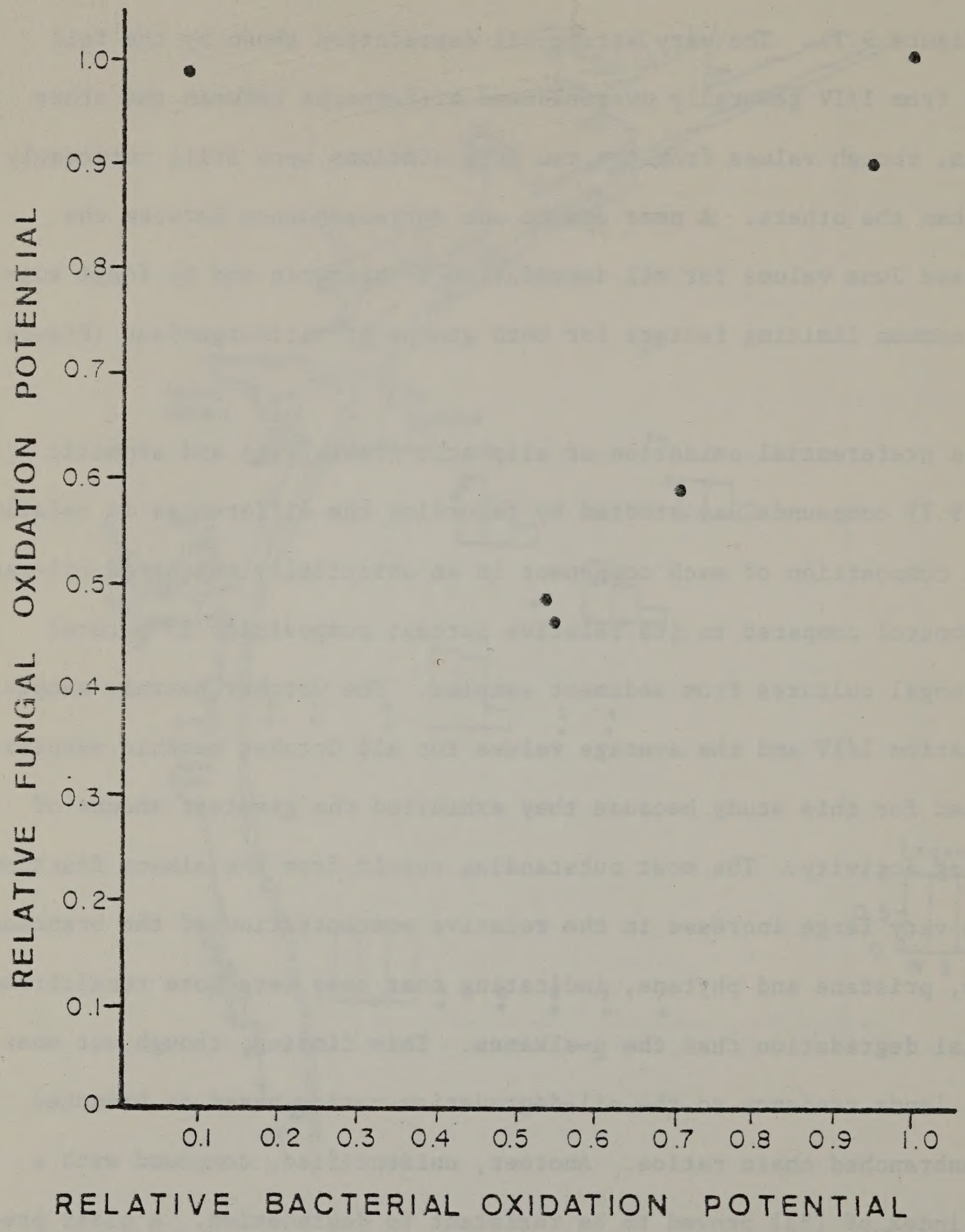

Figure 9.8 Association of Fungal and Bacterial n-alkane Oxidation Potentials in Spring Sediment Samples. The Potentials Were Standardized Relative to the Sample Showing the Maximum Degradation.

TABLE 9.6

PREFERENTIAL DEGRADATION OF SLCO[1] ALKANES BY NATURAL MIXED CULTURES FROM OCTOBER BENTHIC SAMPLES[2]

Kovats Index	Weathered Controls % Composition		Station 1/IV Δ% Composition		Avg. All Stations Δ% Composition	
	0.1% oil	0.5% oil	0.1% oil	0.5% oil	0.1% oil	0.5% oil
1600[a]	8.50	12.30	-3.50	1.70	1.10	1.05
1620	2.30	3.40	-0.60	-0.40	0.98	-0.37
1670[b]	6.10	6.95	29.50	0.45	7.17	-0.88
1700	9.70	10.90	-8.40	0.90	-1.28	0.62
1780[c]	2.85	2.85	6.25	0.45	3.68	0.45
1800	9.55	9.45	-5.35	0.15	-1.27	0.25
1851	3.40	3.20	7.50	0.00	1.62	0.10
1900	9.15	8.30	-3.65	-0.50	-1.15	-0.10
2000	7.55	6.75	-3.25	-0.55	-1.08	-0.05
2100	6.40	5.55	-4.40	-0.25	-1.27	0.15
2200	5.80	5.20	-3.20	-0.20	-1.05	0.18
2300	5.05	4.50	-1.65	-0.30	-0.82	0.10
2400	4.25	3.75	-1.35	-0.35	-0.78	-0.05
2500	3.35	2.95	-3.35	-0.45	-1.17	-0.18
2600	3.40	2.95	-1.90	-0.25	-0.87	-0.10
2700	2.65	2.35	-2.65	-0.15	-0.83	-0.03
2800	2.15	1.65	-2.15	0.05	-1.08	0.05
2900	2.15	1.80	-2.15	0.20	-0.55	0.05
3000	2.20	1.90	-2.20	-0.10	-0.83	-0.40
3100	2.05	1.95	-2.05	-0.35	-0.18	-0.63
3200	1.45	1.45	-1.45	-0.15	0.35	0.32

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[1] Southern Louisiana Crude Oil.

[2] After 45 days in shake culture at 25°C; sediment diluted 1:5 with artificial seawater.

[a] Indices with two final zeros indicate n-alkanes, e.g. 1600 = n-C_{16}.

[b] Pristane, an isolkane.

[c] Phytane, an isolkane.

TABLE 9.7

PREFERENTIAL DEGRADATION OF SLCO[1] AROMATICS BY NATURAL MIXED CULTURES FROM OCTOBER BENTHIC SAMPLES[2]

Kovats Index	Weathered Controls % Composition		Station 1/IV Δ% Composition		Avg. All Stations Δ% Composition		Tentative Component Identification
	0.1% oil	0.5% oil	0.1% oil	0.05% oil	0.1% oil	0.05% oil	
1750	0.00	1.65	0.0	3.05	0.0	3.00	Naphthalene
1870	0.00	11.15	0.0	0.75	7.03	1.38	Methylnaphthalene
1910	0.00	2.45	0.0	2.55	2.06	2.67	Methylnaphthalene
1980	4.50	9.75	2.50	-0.75	3.30	-0.68	Dimethylnaphthalene
2020	9.65	15.95	2.05	1.85	5.28	1.40	Dimethylnaphthalene & Biphenyl
2060	1.50	4.10	5.10	0.40	3.07	0.27	Dimethylnaphthalene & C_3-Naphthalene
2080	1.00	3.95	3.10	-0.95	2.43	-0.73	Dimethylnaphthalene & C_3-Naphthalene
2110	1.00	3.75	2.90	-0.15	2.62	-0.23	C_3-Naphthalene & Methylbiphenyl
2130	18.60	10.00	-1.60	1.12	-5.83	1.10	C_3-Naphthalene & Methylbiphenyl
2170	11.15	7.90	-0.55	-1.20	-1.63	-1.22	C_3-Naphthalene & C_4-Naphthalene
2210	8.20	6.55	0.20	-1.05	-0.77	-1.20	C_3-Naphthalene & C_4-Napththalene
2220	1.70	1.20	-0.10	0.20	-0.05	0.02	
2240	4.40	3.20	-1.00	0.10	-0.47	-0.10	
2290	2.70	1.55	-0.20	-0.35	-0.73	-0.48	Other peaks are mostly unknown at this time.
2310	6.85	3.40	-1.55	-1.20	-3.20	-1.48	
2430	15.80	8.55	-3.60	-2.85	-5.93	-2.98	
2520	13.00	4.85	-7.40	-1.55	-7.07	-0.75	

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[1] Southern Louisiana Crude Oil

[2] After 45 days in shake culture at 25°C; sediment diluted 1:5 with artificial seawater.

for the degradation of aromatic compounds. For example, dimethylnaphthalene appeared to be more susceptible to degradation at the lower oil concentration than at the higher. Naphthalene, methylnaphthalene and dimethylnaphthalene appeared to be relatively recalcitrant. The data suggested that C_3-naphthalene might have been more easily degraded than either its unsubstituted counterpart or naphthalene with an even-numbered side chain. However, most of the metabolic activity was directed toward the unidentified, heavier aromatics with retention indices above 2300.

Pure Cultures

The purpose of these experiments was to evaluate quantitatively the potential of selected fungal isolates to utilize SLCO. The STOCS isolates tested were selected on the basis of their response in the silica-gel-oil assay; each isolate demonstrated the potential to grow in the presence of SLCO. Two yeast and two filamentous forms were examined. The yeast isolates were identified as *Cryptococcus albidus* and *Candida diddensii*; the latter is most frequently found in marine environments. The two filamentous forms selected were *Paecilomyces* sp. and *Nodulosporium* sp. Each organism was tested for its ability to grow and degrade SLCO in the presence or absence of inorganic supplements. The effect of glucose addition to cultures with inorganic supplements and SLCO was also examined.

Stationary-phase cultures grown in a chemically defined medium were used as inocula. RILA Marine Mix (RILA Products, Teaneck, N.J.) hydrated to a 3.5% final concentration was used as a basal solution for the experimental media. Three experimental media were prepared and supplemented as follows: 1) SLCO, 0.1%; 2) SLCO, 0.1%; KNO_3, 50 mM; NH_4Cl, 50 mM; KH_2PO_4, 10 mM; ferric-ammonium citrate, 100 µg/ℓ; ZnCl, 100 µg/ℓ; and 3) SLCO, 0.1%; inorganic salts, as described above; and glucose, 2.0%. For each type of medium tested, each isolate was used to prepare five cultures. All experimental cultures were incubated at 25°C on a reciprocating shaker. At intervals, one culture growing in each type of medium was removed and the amount of growth and oil degradation determined. For the yeast cultures, a 0.1 ml aliquot was removed from the culture for determination of the number of CFU/ml. The remaining culture was subjected to Soxhlet extraction with benzene. The extracts were analyzed by gas chromatography. Cultures of the filamentous isolates were processed as described for the cultures of the yeast isolates except that determination of the number of CFU/ml was not performed. Instead, growth of the filamentous cultures was estimated by dry weight determinations following extraction. Determinations of growth and oil degradation were performed after 0, 5, 10, 20 and 30 days incubation.

Of the two yeast isolates tested, only *C. diddensii* exhibited an increase in the number of CFU/ml during culture in the simple medium supplemented only with SLCO (Figure 9.9; Appendix H, Table 6). Growth (increase in the number of CFU/ml) in the medium supplemented with inorganic salts did not differ significantly from that observed in cultures grown in the simple SLCO medium. In both media there was about a ten-fold increase in the number of CFU/ml during the first days of incubation, after which the number of CFU/ml remained nearly constant. Initially, *C. diddensii* grew at a faster rate in the glucose-supplemented medium than in the simple SLCO medium. Later, however, the number of CFU/ml decreased. In contrast to *C. diddensii*, *C. albidus* was unable to grow in a medium supplemented only with SLCO. In fact, the number of CFU/ml decreased dramatically (Figure 9.9; Appendix H, Table 6). Growth of *C. albidus* in glucose-supplemented medium was similar to that exhibited by *C. diddensii* in the same medium.

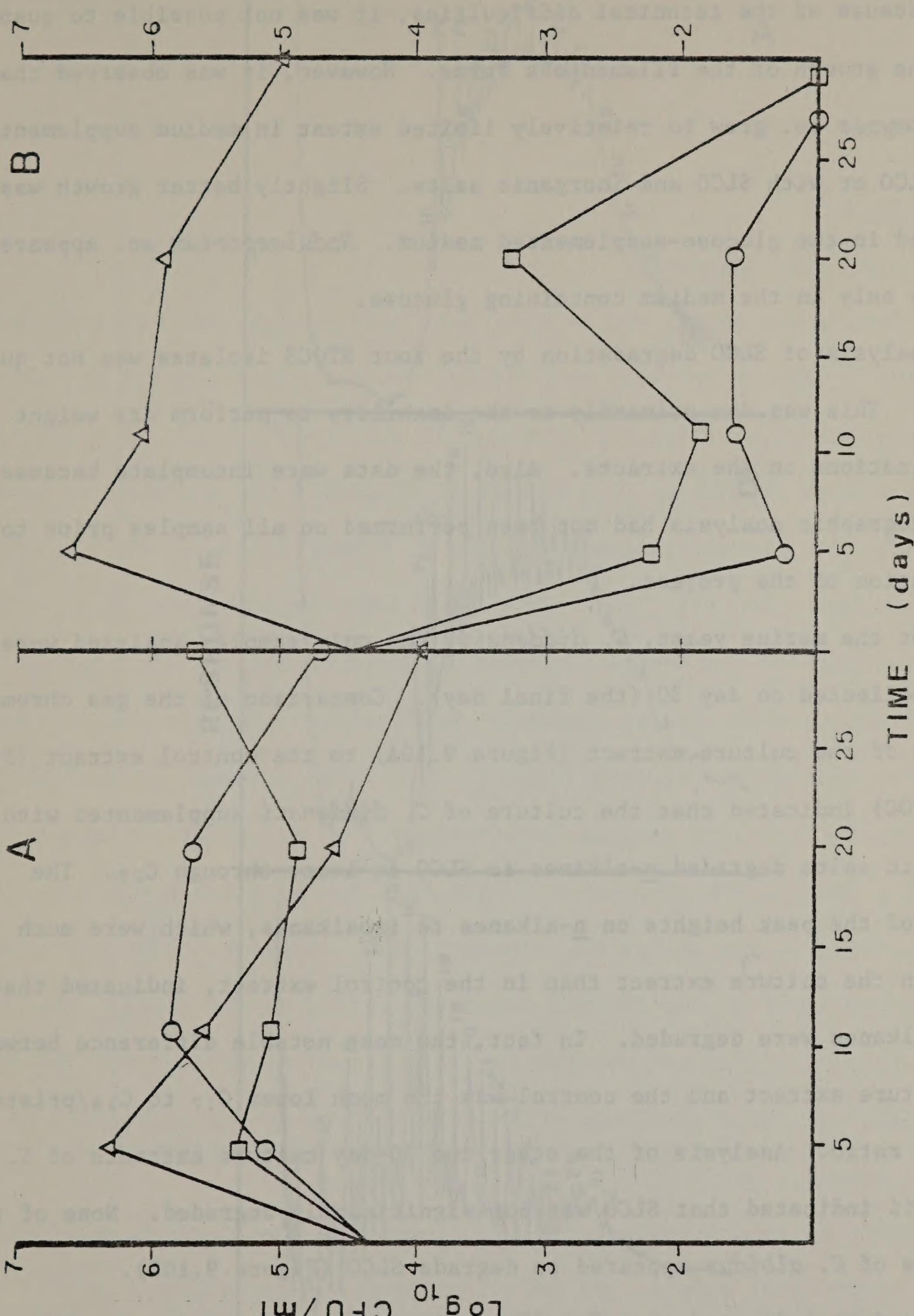

Figure 9.9 Growth of Two Yeast Isolates in Pure Cultures in the Presence of 0.1% v/v Crude Oil (SLCO). Results Presented in A Represent Growth of *Candida diddensii* and Those in B Represent *Cryptococcus albidus*. (○) - SLCO; (□) -SLCO, N, P, Fe, Zn; and (△) - SLCO, N, P, Fe, Zn and Glucose.

Because of the technical difficulties, it was not possible to quantitate the growth of the filamentous forms. However, it was observed that *Paecilomyces* sp. grew to relatively limited extent in medium supplemented with SLCO or with SLCO and inorganic salts. Slightly better growth was observed in the glucose-supplemented medium. *Nodulosporium* sp. appeared to grow only in the medium containing glucose.

Analysis of SLCO degradation by the four STOCS isolates was not quantitative. This was due primarily to the inability to perform dry weight determinations on the extracts. Also, the data were incomplete because gas chromatographic analysis had not been performed on all samples prior to the termination of the project.

For the marine yeast, *C. diddensii*, the only samples analyzed were those collected on day 30 (the final day). Comparison of the gas chromatograms of the culture extract (Figure 9.10A) to the control extract (Figure 9.10C) indicated that the culture of *C. diddensii* supplemented with inorganic salts degraded n-alkanes in SLCO at least through C_{28}. The ratios of the peak heights on n-alkanes to isoalkanes, which were much lower in the culture extract than in the control extract, indicated that the n-alkanes were degraded. In fact, the most notable difference between the culture extract and the control was the much lower C_{17} to C_{18}/pristane-phytane ratio. Analysis of the other two 30-day culture extracts of *C. diddensii* indicated that SLCO was not significantly degraded. None of the cultures of *C. albidus* appeared to degrade SLCO (Figure 9.10B).

The data indicated that *Paecilomyces* sp. was able to degrade SLCO. Extracts of 30-day cultures grown in simple SLCO medium degraded n-alkanes greater than C_{30}, whereas extracts from cultures grown in medium supplemented with inorganic salts degraded n-alkanes through C_{17} to C_{18}. In cultures grown in glucose-supplemented medium, n-alkanes up to C_{17} were degraded by

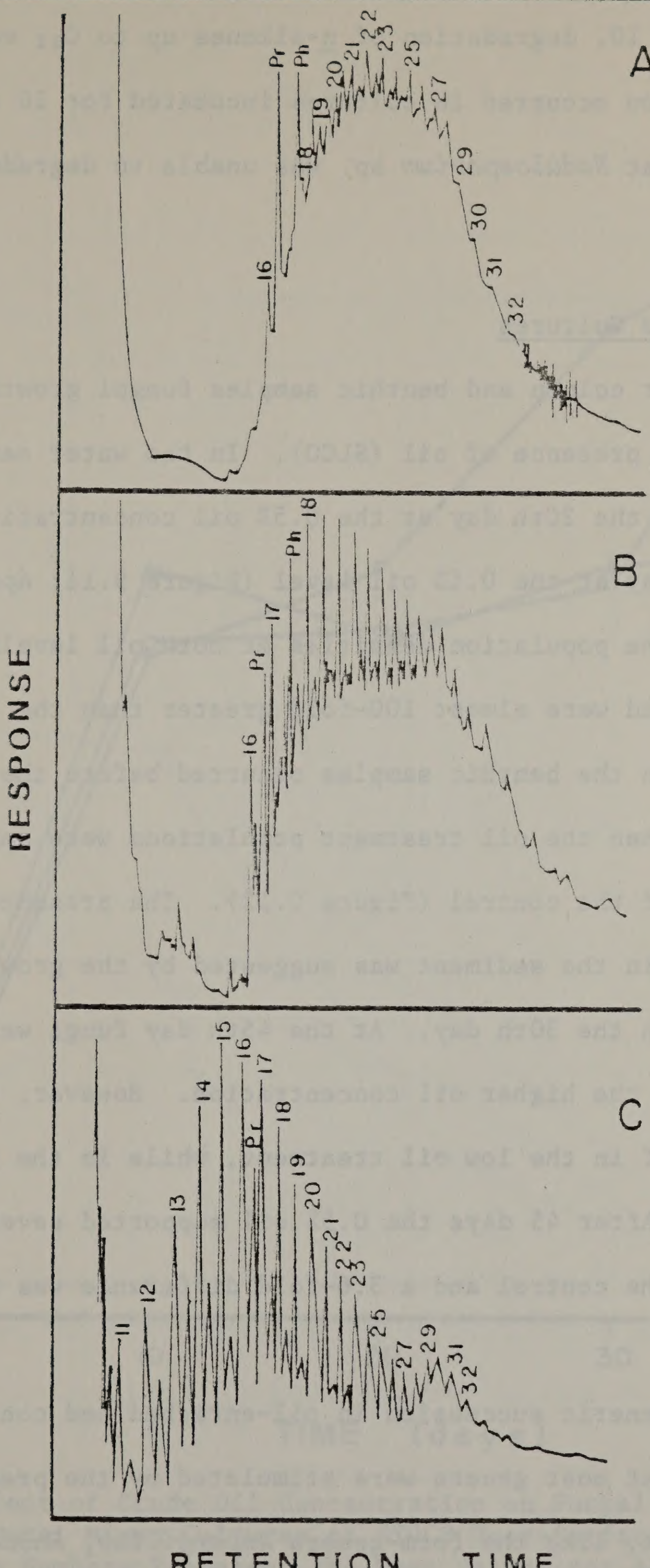

Figure 9.10 Gas Chromatograms of Hexane Extracts from 30-day-old Crude Oil (0.1% SLCO) Enriched Pure Cultures of *Candida diddensii* (A), *Cryptococcus albidus* (B) and an Autoclaved Oil Control (C). No Supplements were Added to the Culture of *Cryptococcus albidus*. The Culture Medium in which *Candida diddensii* was Grown was Supplemented with N, P, Fe and Zn.

day five. At day 10, degradation of n-alkanes up to C_{22} was evident. No further degradation occurred in cultures incubated for 20 and 30 days. The data indicated that *Nodulosporium* sp. was unable to degrade SLCO.

Effects of Oil

Natural Mixed Cultures

In both water column and benthic samples fungal growth was eventually stimulated by the presence of oil (SLCO). In the water samples the effect was noticeable by the 20th day at the 0.5% oil concentration, but was delayed beyond the 30th day at the 0.1% oil level (Figure 9.11; Appendix H, Table 7). By the 40th day the population densities at both oil levels were approximately the same and were almost 100-fold greater than the control. Stimulation of fungi in the benthic samples occurred before the 30th day at both oil levels when the oil treatment populations were approximately equal, but double that of the control (Figure 9.12). The presence of available carbon compounds in the sediment was suggested by the growth of the control population through the 30th day. At the 45th day fungi were still increasing in numbers in the higher oil concentration. However, the numbers had begun to level off in the low oil treatment, while in the control the numbers decreased. After 45 days the 0.5% oil supported seven times the number of fungi in the control and a 3.6-fold difference was maintained by the 0.1% oil level.

A study of generic succession in oil-enriched and control water column samples showed that most genera were stimulated by the presence of oil (Table 9.8). Some, like the form-genera *Aspergillus*, *Rhodotorula* and *Fusarium* predominated in the first two weeks then decreased, whereas others such as *Aureobasidium* appeared after a longer oil exposure. The marine imperfect yeasts *Candida* and *Rhodotorula* actually decreased in frequency after 30 days in the control.

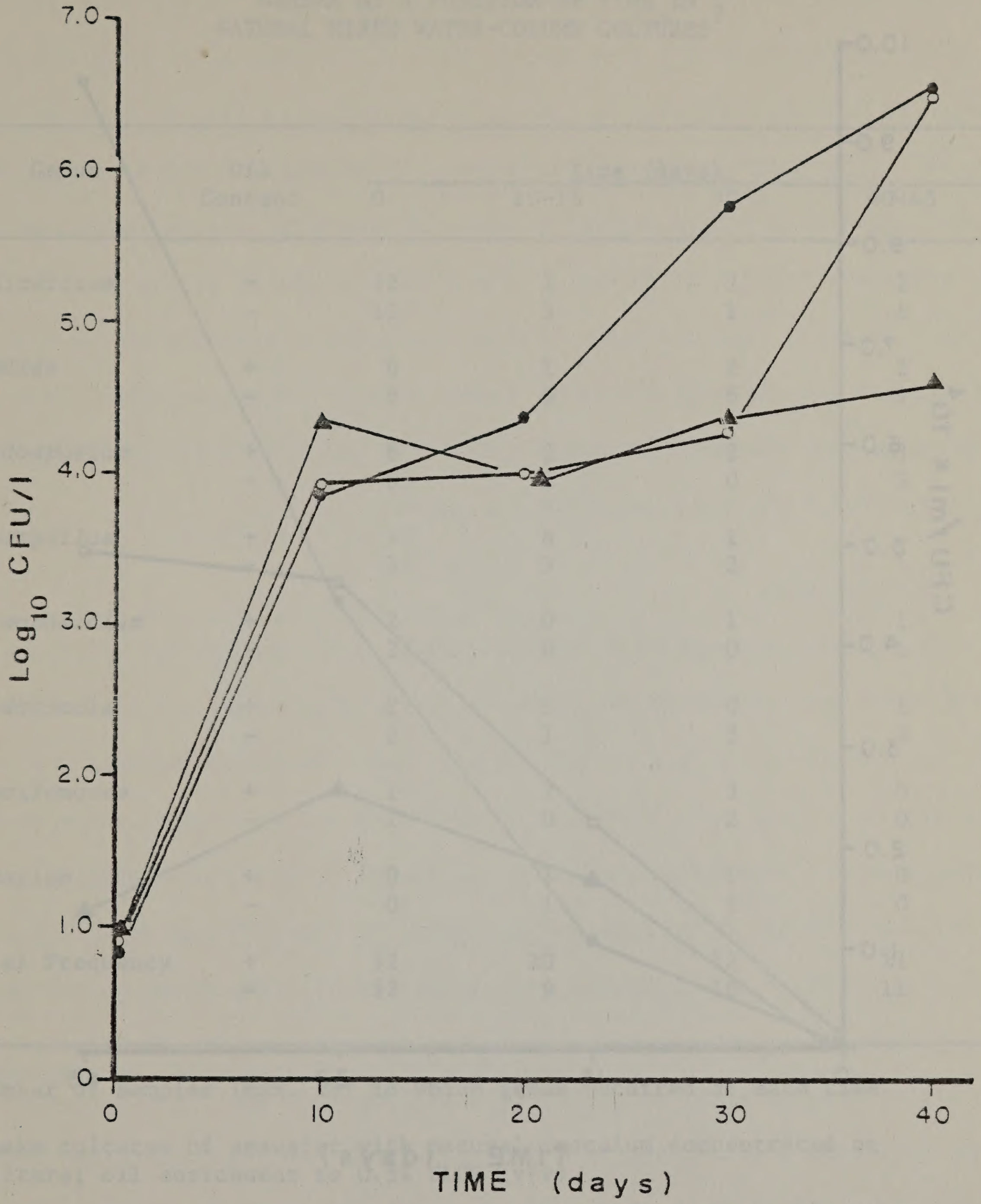

Figure 9.11 Effect of Crude Oil Concentration on Fungal Growth in Natural Mixed Cultures of STOCS Near-Surface Waters. The Numbers Represent the Mean Values for all Samples from the July and August Collections. (●) - 0.5% oil (SLCO); (○) - 0.1% oil (SLCO); (▼) - control to which no oil was added.

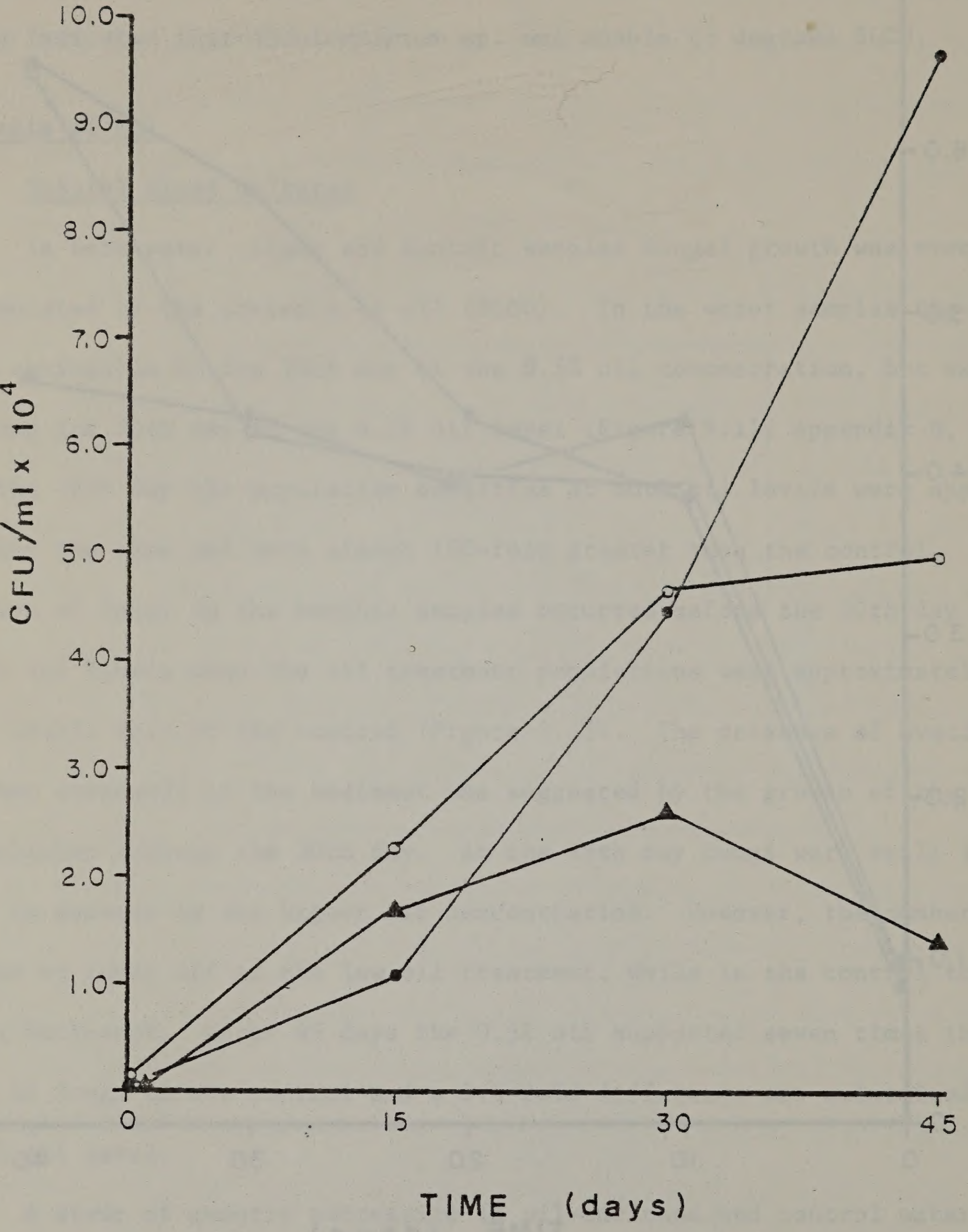

Figure 9.12 Effect of Crude Oil Concentration on Fungal Growth in Natural Mixed Cultures of STOCS Benthic Sediments Diluted (1:5) with Artificial Seawater. The Numbers are the Mean Values for all Samples from the Fall Cruise. (●) - 0.5% oil (SLCO); (○) - 0.1% oil (SLCO); (▼) - control to which no oil was added.

TABLE 9.8

OCCURRENCE[1] OF PREDOMINANT FUNGAL GENERA AS A FUNCTION OF TIME IN NATURAL MIXED WATER-COLUMN CULTURES[2]

Genus	Oil	Time (days)			
	Content	0	10-15	30	40-45
Penicillium	+	12	3	2	2
	-	12	3	1	1
Candida	+	6	1	2	2
	-	6	0	6	3
Cladosporium	+	6	2	2	3
	-	6	1	0	2
Aspergillus	+	3	6	1	2
	-	3	3	2	1
Aureobasidium	+	2	0	1	1
	-	2	0	0	1
Rhodotorula	+	2	3	0	1
	-	2	1	3	3
Paecilomyces	+	1	2	3	0
	-	1	0	2	0
Fusarium	+	0	3	1	0
	-	0	1	2	0
Total Frequency	+	32	20	12	11
	-	32	9	16	11

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[1] Number of samples (max. 18) in which genus occurred at each time.

[2] Shake cultures of seawater with natural inoculum concentrated on filters; oil enrichment to 0.5% SLCO (v/v)

Generic extinction was approximately twice as fast in the benthic controls as in the oil-enriched sediment (Table 9.9). *Aspergillus* and *Fusarium* persisted in the presence of the oil throughout the experiment. The yeast *Candida* performed better in the presence of the oil than in the control. *Cladosporium* appeared relatively frequently in the earlier and later stages of oil degradation in both the water and the sediment samples, but was less frequent at 30 days. The number of genera in the oil-treated benthic cultures remained high, whereas the generic richness of the corresponding control cultures decreased (Table 9.10). In oil-enriched water samples generic richness decreased initially, but then showed a steady increase toward the original number of genera.

Pure Cultures

The growth response of a fungal isolate in the silica-gel-oil tube assay was a good indicator of the stimulatory effect that oil might have on that particular strain in the water column. No growth indicated either toxicity or an isolate's inability to use crude oil as a sole carbon and energy source. Approximately 60% of the isolates tested were able to grow on crude oil (Table 9.11; Appendix H, Table 8). *Candida* and *Penicillium* isolates exhibited predominantly good growth on oil; *Rhodotorula, Cephalosporium* and *Alternaria* isolates also tended to grow well. Form-genera exhibiting predominantly no growth were *Cladosporium* and *Cephalosporium*. Other form-genera responding poorly were *Fusarium* and *Aureobasidium*. Two form-genera, *Aspergillus* and *Paecilomyces*, showed a very uneven response to oil with many isolates exhibiting excellent growth on oil, but with even more isolates showing no growth.

Most of the studies to date on the effect of petroleum on fungi in pure culture have used high densities of log phase inoculum and high levels

TABLE 9.9

OCCURRENCE[1] OF PREDOMINANT FUNGAL GENERA AS A FUNCTION OF TIME IN NATURAL MIXED BENTHIC CULTURES[2]

Genus	Oil Content	Time (days) 0	10-15	30	40-45
Penicillium	+	16	6	3	1
	-	16	6	1	0
Aspergillus	+	11	9	5	4
	-	11	1	3	4
Cladosporium	+	11	7	0	2
	-	11	5	0	0
Candida	+	3	2	3	0
	-	3	0	0	0
Fusarium	+	3	6	5	4
	-	3	5	4	1
Rhodotorula	+	3	1	0	0
	-	3	0	0	0
Paecilomyces	+	2	1	1	1
	-	2	1	1	0
Total Frequency	+	49	37	17	12
	-	49	18	9	5

[1] Number of samples (max. 18) in which genus occurred at each time.

[2] Shake cultures of 20 ml benthic sediments diluted 1:5 with artificial seawater; oil enrichment to 0.5% SLCO (v/v)

TABLE 9.10

EFFECT OF SLCO[1] ON GENERIC RICHNESS IN NATURAL MIXED CULTURES[2]

Time (days)	Benthic		Water Column	
	Oil[a]	Control	Oil[a]	Control
0	8.0	8.0	5.0	5.0
10-15	10.5	5.0	2.4	1.6
30	8.0	4.5	3.6	1.4
40-45	9.5	3.5	3.8	2.2

[1] Southern Louisiana crude oil.

[2] Average number of fungal genera per cruise (2 benthic, 5 water column) in Southern Louisiana Crude-treated and control subsamples.

[a] Oil added at 0.5% (v/v)

TABLE 9.11

EFFECT OF SLCO[1] ON GROWTH OF PREDOMINANT FUNGAL GENERA[2]

Genus	Isolates (%) by Genus			
	Good Growth[a]	Weak Growth[b]	No Growth[c]	Total Isolates Tested
Penicillium	56.0	16.0	28.0	75
Aspergillus	27.1	25.7	47.1	70
Fusarium	6.3	42.8	50.8	63
Cladosporium	3.9	25.5	70.6	51
Candida	74.4	16.3	9.3	43
Cephalosporium	19.2	19.2	61.5	26
Rhodotorula	28.6	52.4	19.0	21
Paecilomyces	31.6	31.6	36.8	19
Alternaria	36.4	45.4	18.2	11
Aureobasidium	0.0	50.0	50.0	10
Total%	30.8	28.0	41.1	

[1] Southern Louisiana Crude Oil.

[2] Oil added to cover one-half of silica-gel slant culture established in presence of limited (0.01%) glucose; growth scored after one month.

[a] Isolates showing good growth (G) above or below oil surface.

[b] Isolates showing only weak growth (g) above or below oil surface.

[c] Isolates showing no growth or toxicity symptoms--above and below oil surface.

of nitrogen and phosphorus. These conditions bear little resemblance to natural conditions in marine waters. The effect of SLCO on *Candida diddensii* in artificial seawater was studied using low densities of starved inoculum and various combinations of the high and low concentrations of nitrogen, phosphorus, carbohydrate and iron present in the STOCS water column (Figure 9.13). Triplicate shaken cultures of the various treatments with and without 0.1% (v/v) oil were sampled periodically and the number of CFU/ml were determined as described for the rate degradation studies. The inoculum consisted of 190 cells/ml (final concentration) starved by three successive subcultures after 24 h in unamended artificial seawater.

Severe oil toxicity was observed in all cases, with survival rates ranging from 0 to 3% of the no-oil control. Maximum toxicity was observed at three days in some treatments and six days in others, but no consistent association between nutrient level and recovery time was discernible. By the 11th day, the cell density of most oil treatments exceeded the no-oil controls and by the 22nd day, oil treatments had from 350 to 650% more cells than the controls. The slow rates of reproduction in the controls and the reduced growth in oil treatments toward the end of the experiment were consistent with the hypothesis that growth was being limited by the rate of autolytic nutrient recycling.

DISCUSSION

STOCS MYCOTA

The high concentrations of fungi recorded in the spring water samples (max. 84 CFU/ml) were quite similar to those reported for March samples from the United States Atlantic continental shelf (Colwell *et al.*, 1976). The spring peak in water column fungi corresponded to the peak spore load of continental air masses resulting from the coincidence of maximum substrate

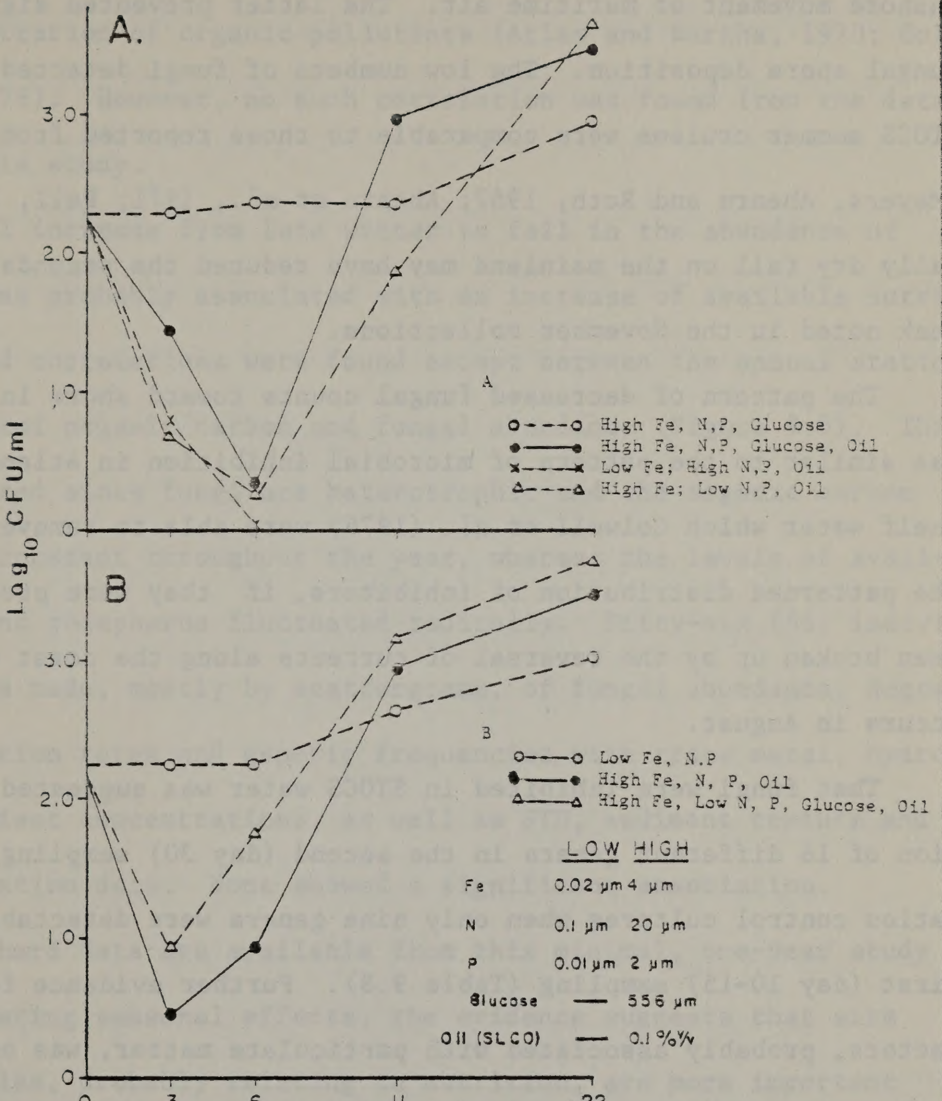

Figure 9.13 Effect of Crude Oil on Growth of *Candida diddensii* in Artificial Seawater Containing High and Low Concentrations of Fe, N, P, and Glucose Found in STOCS Waters.

availability with optimum temperature and moisture conditions. The last continental fronts of the season moved out over the Gulf during this period. The much lower fungal counts during the hot, dry summer months (Figure 9.3) coincided with a period of low air spore loads coupled with the prevailing onshore movement of maritime air. The latter prevented significant new fungal spore deposition. The low numbers of fungi detected during the STOCS summer cruises were comparable to those reported from pelagic waters (Meyers, Ahearn and Roth, 1967; Ahearn *et al*., 1971; Fell, 1967). An unusually dry fall on the mainland may have reduced the secondary abundance peak noted in the November collections.

The pattern of decreased fungal counts toward shore in the July samples was similar to the pattern of microbial inhibition in Atlantic continental shelf water which Colwell *et al*. (1976) were able to remove by heating. The patterned distribution of inhibitors, if they were present, would have been broken up by the reversal of currents along the coast which usually occurs in August.

That fungi were inhibited in STOCS water was suggested by the detection of 16 different genera in the second (day 30) sampling of rate-degradation control cultures when only nine genera were detectable in the first (day 10-15) sampling (Table 9.8). Further evidence for inhibitory factors, probably associated with particulate matter, was noted in the enumeration studies. The greater the dilution of sediment, the greater the estimated number of fungi. Conversely, the greater the concentration of water sample, the lower the estimated number of fungi.

Another source of underestimation of the number of fungal propagules was the 100-fold increase in fungi in the surface film compared to water taken from 10 cm below the surface as reported by Crow *et al*. (1975). Benthic fungi were found to be more than 10^4 times more abundant than detectable water column fungi. This difference was larger than any previously reported.

An inshore to offshore and spring to winter increase of the percentage of hydrocarbonoclastic fungi in the water column is presumably associated with the concentration of organic pollutants (Atlas and Bartha, 1973; Colwell *et al.*, 1976). However, no such correlation was found from the data available in this study.

The general increase from late winter to fall in the abundance of benthic fungi was probably associated with an increase of available nutrients. However, no good correlations were found except between the annual station averages for total organic carbon and fungal abundance (Figure 9.5). This was not unexpected since fungi are heterotrophic and the organic carbon level was very constant throughout the year, whereas the levels of available nitrogen and phosphorus fluctuated radically. Fifty-six (56) individual comparisons were made, mostly by scattergrams, of fungal abundance, degrader ratios, degradation rates and generic frequencies with trace metal, hydrocarbon and nutrient concentrations, as well as STD, sediment texture and bacterial population data. None showed a significant association.

Though no hard data are available from this minimal, one-year study effort for comparing seasonal effects, the evidence suggests that site specific variables, probably relating to nutrition, are more important than seasonal differences (Figure 9.7). The n-alkane degradation rates at the two deep benthic stations remained low relative to the rates at the other stations during all three seasons. The surge in the relative oil-degradation in the October sample from the Rio Grande delta station (1/IV) coincided with the sharp peak in the annual discharge of runoff of agricultural lands from impoundments up river. The effect of oil concentration on the degradation "potential" of benthic and water column samples was predictable and evident from the experimental results. In water column samples, where limiting levels of nitrogen and phosphorus are to be expected, the rate of degradation (utilization) should be independent of oil concentration (Figure 9.11). In the benthic samples, where more nitrogen and phosphorus is available and growth is limited by carbon levels, one expects to see more degradation (utilization) of oil at the higher oil concentrations (Figure 9.10). The preferential degradation of C_3-naphthalene compared to the unsubstituted compound is similar to the results noted by Beam and Perry (1974b) for n-alkyl-substituted cyclo paraffins.

CONCLUSIONS

1. Viable fungal units were much more abundant in the STOCS water column in the spring than in the summer, fall or early winter.

2. The percentage of hydrocarbonoclastic fungi in near-surface waters increased from a low in the spring through the summer to a winter high.

3. The percentage of hydrocarbonoclastic water column fungi increased with the distance from shore.

4. The population density of benthic fungi tended to increase from late-winter through late-spring to a fall maximum.

5. The annual average abundance of benthic fungi at each station was a function of the organic carbon concentration of the sediment.

6. STOCS benthic fungi were generally over one thousand times more abundant than detectable fungi in near-surface waters.

7. Form-species of *Penicillium, Cladosporium* and *Candida* predominated in the water column.

8. Form-species of *Penicillium, Aspergillus, Cladosporium* and *Fusarium* predominated in the benthos.

9. Generic richness of the water mycota decreased during the warm months.

10. The taxonomic composition of the STOCS mycota, with the exception of the yeasts, was similar to that of continental air spora.

11. The fungal n-alkane degradation potentials of STOCS waters and sediments tended to decrease offshore across the shelf, especially during the summer.

12. Fungal n-alkane degradation potential was greatly increased in the presence of freshwater outwash.

13. Conditions limiting bacterial oil-degradation potential in June also limited fungal oil-degradation.

14. Fungi in pure and natural mixed cultures preferentially utilized n-alkanes of intermediate chain length.

15. Isoalkanes were relatively resistant to fungal degradation.

16. Naphthalene appeared to be more recalcitrant to fungal degradation than was its C_3-substituted counterpart.

17. Crude oil (SLCO) toxicity to a petroleum-degrading yeast, *Candida diddensii*, increased during the first few days after application.

18. Natural mixed fungal cultures were generally stimulated by SLCO after a lag period of one to four weeks.

19. Form-species of *Penicillium*, *Aspergillus*, *Cladosporium* and *Fusarium* were frequently encountered after two weeks in oil-enriched water and sediment samples.

20. Crude oil increased the generic richness of natural mixed cultures over that of the controls.

21. A majority of the isolates of *Candida* and *Penicillium* tested in pure culture grew well on whole oil.

LITERATURE CITED

Ahearn, D. G., S. P. Meyers, and P. G. Standard. 1971. The role of yeasts in the decomposition of gills in the marine environment. Dev. Ind. Microbiol. 12:126-134.

________., F. J. Roth, Jr., and S. P. Meyers. 1968. Ecology and characterization of yeasts from aquatic regions of South Florida. Mar. Biol. (N.Y.) 1:291-308.

__________. 1962. A comparative study of marine and terrestrial strains of *Rhodoturola*. Can J. Microbiol. 8:121-131.

Alderman, D. J. 1976. Fungal diseases of marine animals. pp 223-260. *In* E. B. G. Jones (ed.), Recent advances in aquatic mycology. John Wiley and Sons, N.Y.

Andrews, J. H. 1977. Observations on the pathology of seaweeds in the Pacific Northwest. Can J. Bot. 55:1019-1027.

Atkins, D. 1954. Further notes on a marine member of the Saprolegniaceae, *Leptolegnia marina* n. sp. infecting certain invertebrates. J. Mar. Biol. Assoc. U.K. 33:613-625.

Atlas, R. M., A. Horowitz and M. Busdosh. 1978. Prudhoe crude oil in arctic marine ice, water and sediment ecosystems: Degradation and interactions with microbial and benthic communities. J. Fish. Res. Board Can. 35:585-590.

Atlas, R. M. and R. Bartha. 1973. Abundance, distribution and oil biodegradation potential of microorganisms in Raritan Bay. Environ. Pollut. 4:291-300.

____________., 1972a. Biodegradation of petroleum in seawater at low temperatures. Can. J. Microbiol. 18:1851-1855.

____________., 1972b. Degradation and mineralization of petroleum in seawater: Limitation by nitrogen and phosphorus. Biotechnol. Bioeng. 14:309-318.

Bahnweg, G., and F. K. Sparrow. 1974. Four new species of *Thraustochytrium* from Antarctic regions with notes on the distribution of zoosporic fungi in the marine ecosystems. Am. J. Bot. 61:754-766.

Barghoorn, E. S. 1942. The occurrence and significance of marine cellulose-destroying fungi. Sci. (Wash. D. C.) 96:358-359.

Beam, H. W. and J. J. Perry. 1974a. Microbial degradation of cycloparafinnic hydrocarbons via co-metabolism and commensalism. J. Gen. Microbiol. 82:163-169.

____________., 1974b. Microbial degradation and assimilation of n-alkyl-substituted cycloparaffins. J. Bacteriol. 118:394-399.

Bell, G. H. 1971. The action of monocarboxylic acids on *Candida tropicalis* growing on hydrocarbon substrates. Antonie Van Leeuwenhoek J. Microbiol. Serol. 37:385-400.

Blumer, M., and J. Sass. 1972. Oil pollution: persistence and degradation of spilled fuel oil. Sci. (Wash. D.C.) 176:1120-1122.

________., G. Souza and J. Sass. 1970. Hydrocarbon pollution of edible shellfish by an oil spill. Mar. Biol. (N.Y.) 5:195-202.

Borut, S. Y., and T. W. Johnson, Jr. 1962. Some biological observations on fungi in estuarine sediments. Mycologia 54:181-193.

Brown, R. A., and H. L. Huffman. 1976. Hydrocarbons in open ocean waters. Sci. (Wash. D.C.) 191:847-849.

Buck, J. D., D. G. Ahearn, F. J. Roth, Jr., and S. P. Meyers. 1963. Inhibition of yeasts by a marine bacterium. J. Bacteriol. 85:1132-1135.

Byrne, P. J., and E. B. G. Jones. 1975. Effect of salinity on the reproduction of terrestrial and marine fungi. Trans. Br. Mycol. Soc. 65: 185-200.

Cofone, J., J. D. Walker and J. J. Cooney. 1973. Utilization of hydrocarbons by *Cladosporium resinae*. J. Gen. Microbiol. 76:243-246.

Colwell, R. R., J. D. Walker, B. F. Conrad, and P. A. Seesman. 1976. Microbiological studies of Atlantic Ocean water and sediment from potential offshore drilling sites. Dev. Ind. Microbiol. 17:269-282.

Combs, T. J., R. A. Murchelano, and F. Jurgen. 1971. Yeasts isolated from Long Island Sound. Mycologia 63:178-181.

Conrad, B. F., J. D. Walker, and R. R. Colwell. 1976. Utilization of crude oil and mixed hydrocarbon substrate by Atlantic Ocean microorganisms. Dev. Ind. Microbiol. 17:283-291.

Cook, W. L., J. K. Massey, and D. G. Ahearn. 1973. The degradation of crude oil by yeasts and its effect on *Lebistes reticulatus*. pp 279-282. *In* D. G. Ahearn and S. P. Meyers (eds.), The microbial degradation of oil pollutants. Louisiana State University, Baton Rouge.

Cooney, J. J., and J. D. Walker. 1973. Hydrocarbon utilization by *Cladosporium resinae*. pp. 237-242. *In* D. G. Ahearn and S. P. Meyers (eds.) The Microbial degradation of oil pollutants. Louisiana State University Baton Rouge.

Copeland, B. J., and T. J. Bechtel. 1971. Species diversity and water quality in Galveston Bay, Texas. Water, Air, Soil Pollut. 1:89-93.

Crow, S. A., D. G. Ahearn, W. L. Cook, and A. W. Bourquin. 1975. Densities of bacteria and fungi in coastal surface films as determined by a membrane absorption procedure. Limnol. Oceanogr. 20:644-646.

Davavin, I. A., G. O. Mironov, and I. M. Tsimbal. 1975. Influence of oil on nucleic acids of algae. Mar. Pollut. Bull. 6:13-15.

Davis, H. C., W. H. Weston, and C. Martin. 1954. A fungus disease in clam and oyster larvae. Sci. (Wash. D. C.) 120-36-38.

Dawe, L. L., and W. R. Penrose. 1978. "Bactericidal" property of seawater: Death or debilitation? Appl. Environ. Microbiol. 35:829-833.

Dibble, J. T., and R. Bartha. 1976. Effect of iron on the biodegradation of petroleum in seawater. Appl. Environ. Microbiol. 31:544-550.

Fell, J. W. 1976. Yeast in oceanic regions. pp. 93-124. *In* E. B. G. Jones (ed.), Recent advances in aquatic mycology. John Wiley and Sons, N.Y.

________., 1967. Distribution of yeasts in the Indian Ocean. Bull. Mar. Sci. 17:454-470.

________., D. G. Ahearn, S. P. Meyers, and F. J. Roth. 1960. Isolation of yeasts from Biscayne Bay, Florida and adjacent benthic areas. Limnol. Oceanogr. 5:366-371.

Ganaros, A. E. 1957. Marine fungus infecting eggs and embryos of *Urosalpinx cinerea*. Sci. (Wash. D. C.) 125:1194.

Gibbs, C. F., and S. J. Davis. 1976. The rate of microbial degradation of oil in a beach gravel column. Microbiol. Ecol. 3:55-64.

Gray, W. D., P. C. Pinto, and S. G. Pathak. 1963. Growth of fungi in seawater medium. Appl. Microbiol. 11:501-505.

Griffin, L. F., and J. A. Calder. 1977. Toxic effect of water soluble fractions of crude, refined and weathered oils on the growth of a marine bacterium. Appl. Environ. Microbiol. 33:1092-1096.

Hughes, G. C. 1975. Studies of fungi in oceans and estuaries since 1961: I. Lignicolous, caulicolous, and folicolous species. Oceanogr. Mar. Biol. Ann. Rev. 13:69-180.

Jannash, H. W. 1970. Threshold concentrations of carbon sources limiting bacterial growth in seawater. pp. 321-328. *In* D. W. Hood (ed.), Organic matter in natural waters. University of Alaska Press, College.

Johnson, T. W., Jr., and F. K. Sparrow, Jr., 1961. Fungi in oceans and estuaries. J. Cramer, N.Y.

________., 1958. A fungus parasite in ova of the barnacle *Chthamalus fragilis denticulata*. Biol. Bull. 114:205-214.

Jones, E. B. G. 1976. Lignicolous and algicolous fungi. pp. 1-49 *In* E. B. G. Jones (ed.), Recent advances in aquatic mycology. John Wiley and Sons, N.Y.

Jones, J. G., and M. A. Edington. 1968. An ecological survey of hydrocarbon-oxidizing microorganisms. J. Gen. Microbiol. 52:381-390.

Komagata, K., T. Nakase, and M. Katsuya. 1964. Assimilation of hydrocarbons by yeasts, preliminary screening. J. Gen. Appl. Microbiol. 10:313-321.

Le Petit, J., M. H. N'Gyen and L. Deveze. 1970. Etude de l'intervention des levures dans la bio-degradation en mer des hydrocarbures. Ann. Inst. Pasteur (Paris) 118:709-720.

Ledet, E. J., and J. L. Laseter. 1974. Alkanes at the air-sea interface from offshore Louisiana and Florida. Sci. (Wash. D. C.) 186:261-265.

Llanos, C., and A. Kjoller. 1976. Changes in the flora of soil fungi following oil waste application. Oikos, 27:377-382.

Lowery, C. E., Jr., J. W. Foster, and P. Jurtshuk. 1968. The growth of various filamentous fungi and yeasts on n-alkanes and ketones. I. Studies on substrate specificity. Arch. Microbiol. 60:246-254.

Markovetz, A. J., Jr., J. Cazin, and J. E. Allen. 1968. Assimilation of alkanes and alkenes by fungi. Appl. Microbiol. 16:487-489.

Meyers, S. P., D. G. Ahearn, W. Gunkel, and F. J. Roth, Jr., 1967. Yeasts from the North Sea. Mar. Biol. 1:118-123.

_________., D. G. Ahearn, and F. J. Roth, Jr. 1967. Mycological investigations of the Black Sea. Bull. Mar. Sci. 17:556-596.

Morita, R. Y. 1965. The physical environment of fungal growth: 2. hydrostatic pressure. *In* G. C. Ainsworth and A. S. Sussman (eds.), The fungi: an advanced treatise, Vol. I. the fungal cell. Academic Press, New York.

_________., and C. E. Zobell. 1955. Occurrence of bacteria in pelagic sediments collected during the mid-Pacific expedition. Deep-Sea Res. 3:66-73.

National Academy of Science Workshop. 1975. Petroleum in the marine environment. NAS, Washington, D. C.

Norkrans, B. 1966. Studies on marine occurring yeasts: growth related to pH, NaCl concentration and temperature. Arch. Microbiol. 54:274-392.

Nyns, E. J., J. Brand and A. L. Wiaux. 1968. Assay of the property of fungi to assimilate hydrocarbons by a simple test tube technique. J. Fuel Sci. 48:449-453.

Pady, S. M., and L. Kapica. 1955. Fungi in air over the Atlantic Ocean. Mycologia 47:34-50.

Perry, J. J., and C. E. Cerniglia. 1973a. Studies on the degradation of petroleum by filamentous fungi. pp. 89-94. *In* D. G. Ahearn, and S. P. Meyers (eds.), The microbial degradation of oil pollutants. Louisiana State University, Baton Rouge.

_____________., 1973b. Crude oil degradation by filamentous fungi. J. Gen. Appl. Microbiol. 19:151-153.

Pitts, G. Y., and G. T. Cowley. 1974. Mycoflora of the habitat and midgut of the fiddler crab, *Uca pugilator*. Mycologia 66:669-675.

Ray, S. M., and A. C. Chandler. 1955. *Dermocystidium marinum*, a parasite of oysters. Exp. Parasitol. 4:172-200.

Riddell, R. W. 1950. Permanent stained mycology preparation obtained by slide cultures. Mycologia 42:265-270.

Roth, F. J., P. A. Orpurt, and D. G. Ahearn. 1964. Occurrence and distribution of fungi in a subtropical marine environment. Can. J. Bot. 42:375-383.

Schwarz, J. R., J. D. Walker, and R. R. Colwell. 1974. Hydrocarbons degradation at ambient and *in situ* pressure. Appl. Microbiol. 28:982-986.

Seki, H., and J. Fulton. 1969. Infection of marine copepods by *Metschnikowia* sp. Mycopath. Mycol. Appl. 38:6170.

Siepmann, R., and T. W. Johnson, Jr. 1960. Isolation and culture of fungi from wood submerged in saline and fresh waters. Jour. Elisha Mitchell Sci. Soc. 76:150-154.

Sparrow, F. K., Jr. 1937. The occurrence of saprophytic fungi in marine muds. Biol. Bull. 73:242-248.

_________. 1936. Biological observations on the marine fungi of Woods Hole waters. Biol. Bull. 70:236-263.

Sproston, N. G. 1944. *Ichthyosporidium hoferi* (Plehn and Mulsow, 1911), an internal fungoid parasite of the mackerel. J. Mar. Biol. Assoc. U.K. 26:72-98.

Steele, C. W. 1967. Fungus populations in marine waters and coastal sands of the Hawaiian, Line and Phoenix Islands. Pacific Sci. 21:317-331.

Turner, W. E., and D. G. Ahearn. 1970. Ecology and physiology of yeasts of an asphalt refinery and its watershed. pp. 113-135 *In* D. G. Ahearn (ed.), Recent trends in yeast research. Georgia State University, Atlanta.

Van Uden, N. 1967. Occurrence and origin of yeasts in estuaries. pp. 306-310. *In* G. H. Lauff (ed.) Estuaries. AAAS, Wash., D. C.

_________., and R. Castelo-Branco. 1963. Distribution and population densities of yeast species in Pacific water, air, animals and kelp off southern California. Limnol. Oceanogr. 8:323-329.

Walker, J. D., R. R. Colwell, and L. Petrakis. 1976. Biodegradation rates of components of petroleum. Can. J. Microbiol. 22:1209-1213.

Walker, J. D., and R. R. Colwell. 1975. Some effects of petroleum on estuarine and marine microorganisms. Can. J. Microbiol. 21:305-313.

Walker, J. D. and J. J. Cooney. 1975. Effects of poorly metabolized hydrocarbons on substrate oxidation by *Cladosporium resinae*. J. Appl. Bacteriol. 39:189-195.

________., H. F. Austin, and R. R. Colwell. 1975. Utilization of mixed hydrocarbon substrate by petroleum-degrading microorganisms. J. Gen. Appl. Microbiol. 21:27-39.

________., and R. R. Colwell. 1974a. Microbial degradation of model petroleum at low temperatures. Microb. Ecol. 1:63-95.

___________., 1974b. Microbial petroleum degradation: use of mixed hydrocarbon substrates. Appl. Microbiol. 27:1053-1060.

Watson, A. G., and E. J. Ford. 1972. Soil fungistasis-a reappraisal. Ann. Rev. Phytopathol. 10:327-348.

Wilhm, J. L., and T. C. Dorris. 1966. Species diversity of benthic macroinvertebrates in a steam receiving domestic and oil refinery effluents. Am. Middl. Nat. 76:427-449.

Zobell, C. E., and J. F. Prokop. 1966. Microbial oxidation of mineral oils in Barataria Bay bottom deposits. Z. Allg. Mikrobiol. 6:143-162.

CHAPTER TEN

BENTHIC BACTERIOLOGY

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ABSTRACT

Selected characteristics of benthic bacteria of the South Texas Outer Continental Shelf (STOCS) were measured. Populations of total aerobic heterotrophic bacteria ranged from 4.6×10^4 to 1.3×10^6/ml wet sediment. Hydrocarbon degrading bacteria ranged from 8.0×10^1 to 1.1×10^5/ml wet sediment. The percentage of bacteria in the population able to degrade hydrocarbons was from 0.10 to 20.68%. Bacterial populations exhibited both seasonal and spatial variations.

The predominant genera of aerobic heterotrophic bacteria were *Bacillus*, *Vibrio* and *Pseudomonas*. Hydrocarbon degrading bacteria were predominantly species of *Pseudomonas*.

Oil biodegradation by natural sediment populations ranged from 0 to 91.6%. Low molecular weight n-paraffins were utilized to a greater extent than high molecular weight paraffins. Degradation rates exhibited seasonal variations, but were evenly distributed geographically. Four pure cultures of hydrocarbon degrading bacteria removed from 6.1 to 51.0% of the C_{14}-C_{32} n-paraffins. The maximum degradation rate of n-[1-^{14}C] hexadecane by pure cultures of hydrocarbon degrading bacteria was 0.03 µg/day.

The addition of oil to sediment increased bacterial populations, and changed the composition of the population to one more capable of degrading oil.

INTRODUCTION

A total of 6 x 10^6 metric tons of petroleum hydrocarbons enter the marine environment annually (McAuliffe, 1976). Marine transportation and industrial/municipal waste disposal contribute the majority of oil entering the oceans (McAuliffe, 1976; Wardley Smith, 1976). Two additional sources of hydrocarbons in the marine environment include natural seepage and offshore oil production. The latter source presently contributes less than 3% of the total hydrocarbons in the oceans (Grossling, 1976).

Oil on the sea surface spreads rapidly, increasing the reactive surface area for physical, chemical and biological processes. Evaporation removes many of the volatile components of oil at the sea surface, the rate dependent on weather conditions and oil type (Floodgate, 1972). Oil on the sea surface is also subject to destruction by photooxidation (Klein and Pilpel, 1974). Water soluble components of oil are leached, leaving a heavy residue that may sink to the bottom (Nelson - Smith, 1970). The presence of large quantities of suspended matter and ingestion-egestion by filter feeders may increase the rate at which oil sinks to the bottom (Butler *et al.*, 1976).

Microorganisms are active in degrading petroleum hydrocarbons in the marine environment and are most active in estuarine waters (Lee and Ryan, 1976). Numbers of hydrocarbon degrading bacteria in the marine environment likewise are most numerous near land, where their numbers range from 0.1 to > 10^8/ml sediment or seawater (Zobell, 1969).

Degradation rates of petroleum in the marine environment are dependent on a number of factors: 1) chemical composition of the oil; 2) number and types of microorganisms present; and 3) environmental parameters, including nutrient concentration and temperature. The dependence of oil degradation on these factors is discussed below.

The n-paraffins of crude oils are the most susceptible to microbial degradation (Mechalas *et al.*, 1973; Lee and Ryan, 1976). Among the n-paraffins, the lower molecular weight compounds are degraded most rapidly. The aromatics and cycloalkanes of crude oils are the most resistant to microbial breakdown (Zobell, 1969; Mechalas *et al.*, 1973; Lee and Ryan, 1976). Since different crude oils contain different proportions of n-paraffins, aromatics, and cycloalkanes, they would be expected to undergo different rates of breakdown (Lee, 1976).

A single microbial species is not capable of significantly degrading crude oil, because each species of hydrocarbon degrading bacteria usually attacks only selected crude oil hydrocarbons (Zobell, 1973). Some microorganisms degrade primarily n-paraffins, while others preferentially oxidize aromatics, or attack a wider range of hydrocarbons (Soli, 1973).

Two important environmental parameters that limit oil degradation in the marine environment are nutrient concentration and temperature. The addition of nitrogen and phosphorus to seawater significantly increases oil degradation rates (Atlas and Bartha, 1972a). Low temperatures decrease oil degradation rates and increase the period preceding degradation (Atlas and Bartha, 1972b). Other environmental parameters that regulate the degradation rate of oil in the marine environment are oxygen concentration, oil dispersion, salinity, turbulence, organic matter concentration, oil concentration, and microbial predators (Zobell, 1973).

Degradation of oil by marine microorganisms has been demonstrated extensively. The effects of oil on marine microorganisms, however, have received comparatively little attention. Hood *et al.* (1975) compared benthic bacteria from an oil field salt marsh with those from a pristine salt marsh. Although there was no significant difference in total aerobic heterotrophic bacteria, the number and percent hydrocarbon degrading bacteria were significantly greater in the oil field salt marsh. The authors suggested that the presence of oil altered the relative abundance of the predominant aerobic heterotrophic bacteria.

Walker *et al.* (1974a) examined the effects of South Louisiana crude oil and No. 2 fuel oil on the growth of estuarine bacteria in laboratory cultures. The addition of oil to cultures inhibited the growth of lipolytic, proteolytic, and chitinolytic bacteria during the first seven days incubation. Walker and Colwell (1976c) compared populations of hydrocarbon degrading bacteria from an oil-contaminated area with those from an oil-free area. Numbers of hydrocarbon degrading bacteria and oil biodegradation potential were higher in the oil-contaminated area. Sediment bacteria from the oil-contaminated area were stimulated by oil added to laboratory cultures, while sediment bacteria from the oil-free area were inhibited by added oil.

The effects of four oils (Louisiana crude, Kuwait crude, No. 2 fuel oil, Bunker C oil) on the heterotrophic uptake and mineralization of D-glucose-^{14}C were examined by Hodson *et al.* (1977). The four oils inhibited D-glucose uptake and mineralization. The degree of inhibition by oil was dependent on both the oil type and concentration.

Selected characteristics of benthic bacteria of the South Texas Outer Continental Shelf (STOCS) were measured during 1977. No studies similar to the one described below have been conducted on the benthic bacteria of the STOCS. However, a study comparable to the present one was conducted by Gunkel (1973) in the North Sea.

Data collected at Stations 1, 2 and 3 on all four transects during three seasons (winter, spring and fall) included: 1) number of aerobic heterotrophic and hydrocarbon degrading bacteria; 2) percent hydrocarbon degrading bacteria; 3) predominant genera of aerobic heterotrophic and hydrocarbon degrading bacteria; and 4) oil biodegradation potential. Laboratory studies included: 1) degradation of crude oil and radiolabeled hexadecane by pure cultures of sediment bacteria, and 2) effects of crude oil on the growth and composition of sediment bacteria.

MATERIALS AND METHODS

The crude oil used in this study was South Louisiana crude oil (SLCO). SLCO is produced in the Gulf of Mexico and has been frequently employed in biodegradation and effects studies.

Sediment was collected with a Smith-McIntyre grab at Stations 1, 2 and 3 on all four transects during winter, spring and fall (4-8 March, 13-15 June, and 15-18 October, respectively). The top centimeter of sediment was removed with a spatula and placed in a sterile 150 ml beaker. After mixing, 10 ml sediment subsamples were removed for bacteriological analyses.

Bacterial analyses were initiated immediately after collection of sediment to minimize alteration of bacterial numbers and activity. Procedures initiated immediately included: 1) inoculation of plates and tubes with sediment dilutions for subsequent enumeration and isolation of total aerobic heterotrophic and hydrocarbon degrading bacteria; 2) inoculation of flasks with sediment for oil biodegradation studies; and 3) inoculation of flasks with sediment for oil effects studies. All inoculated plates, tubes, and flasks were secured for subsequent transfer to the laboratory.

Enumeration of Sediment Bacteria

Total Aerobic Heterotrophic Bacteria

Duplicate 10 ml sediment subsamples were diluted 1:10 in artificial seawater (INSTANT OCEAN, Aquarium Systems, Inc., Wickliffe, Ohio) and mixed by swirling for one minute. The resulting suspension was serially diluted in artificial seawater and three dilutions plated in triplicate on Marine Agar 2216 (DIFCO). Bacterial colonies were enumerated with a Quebec Colony Counter after 10 days incubation at the seasonal *in situ* temperature.

Contamination of Marine Agar 2216 medium due to manipulation of this procedure was not a problem. Less than five bacteria appeared per uninoculated plate.

Hydrocarbon Degrading Bacteria

The most probable number (MPN) technique as described by Gunkel (1973) in the three-tube series was used for the enumeration of hydrocarbon degrading bacteria. Duplicate 10 ml sediment subsamples were serially diluted in artificial seawater and one milliliter of four sediment dilutions was placed in tubes containing 10 ml artificial seawater and 0.5 ml autoclaved SLCO. The artificial seawater was supplemented with 1 g NH_4NO_3 and 1 g KH_2PO_4 per liter. The pH of the medium was adjusted to 7.6 by the addition of 1N NaOH. After inoculation, tubes were incubated stationary for 30 days at the seasonal *in situ* temperature. Positive tubes were determined by visual observation of growth.

Control tubes without oil were incubated at the lowest MPN dilution to assure that growth was due to decomposition of oil and not due to nutrients in the sediment inoculum. Growth in control tubes occurred infrequently and was slight compared to growth in tubes containing oil.

Total aerobic heterotrophic and hydrocarbon degrading bacteria were enumerated as described above. The mean number of hydrocarbon degrading bacteria per milliliter wet sediment was divided by the mean number of aerobic heterotrophic bacteria per milliliter wet sediment. The ratio obtained was multiplied by 100 to obtain the percent hydrocarbon degrading bacteria.

Predominant Genera of Aerobic Heterotrophic and Hydrocarbon Degrading Bacteria

Aerobic heterotrophic bacteria were selected from Marine Agar 2216 medium on the basis of colonial morphology. Isolates were maintained for subsequent identification and use in pure culture studies.

Marine Agar 2216 medium was inoculated with growth from MPN oil tubes for isolation of hydrocarbon degrading bacteria. Isolated colonies were inoculated into fresh oil tubes. Bacteria exhibiting growth in fresh oil medium were considered hydrocarbon degraders. These isolates were maintained for subsequent identification and use in pure culture studies. Bacterial isolates were identified to genus level according to *Bergey's Manual of Determinative Bacteriology*, 8th edition.

Oil Biodegradation and Effects Studies

Artificial seawater with *in situ* concentrations of nitrogen and phosphorus (referred to subsequently as basal medium) was employed in all the oil biodegradation and effects studies described below. Nitrogen concentrations used ranged from 0.01 to 1.2 mg NH_4NO_3 per liter, while levels of phosphorus used ranged from 0.003 to 0.1 mg KH_2PO_4 per liter. These nitrogen and phosphorus levels were representative of nutrient concentrations in STOCS bottom water (Sackett *et al.*, 1976).

Pure cultures of aerobic heterotrophic and hydrocarbon degrading bacteria were used in oil biodegradation and effects studies at a cell density of 10^4- 10^5 cells/ml and 10^3 - 10^4 cells/ml respectively. These cell densities were representative of bacterial populations in STOCS sediment as determined by this study.

Biodegradation by Natural Sediment Populations

Duplicate 10 ml sediment subsamples were diluted 1:10 in basal medium containing SLCO. The oil concentration used during winter and spring was 0.5% V/V. SLCO concentration was decreased to 0.05% V/V during fall. Autoclaved oil was used during winter, but subsequent gas chromatographic analysis revealed that the autoclaving process removed a significant fraction of the lower molecular weight n-paraffins. For this reason, unautoclaved oil was used during spring and fall.

Diluted sediment was incubated stationary at the seasonal *in situ* temperature for eight weeks and then frozen until extraction and gas chromatographic analysis. The extraction and gas chromatographic procedures used have been described in Chapter 5 of this report (Giam, 1978).

Duplicate zero-time and weathering controls were included for each season. Zero-time controls were inoculated with 10 ml sediment subsamples from Station 1/I and frozen immediately. Uninoculated weathering controls were incubated with experimental flasks at the seasonal *in situ* temperature.

Biodegradation by Four Pure Cultures of Hydrocarbon Degrading Bacteria

One milliliter of a 48-hour pure culture of hydrocarbon degrading bacteria was added to duplicate flasks containing 98.95 ml basal medium and 0.05 ml unautoclaved SLCO. Flasks were incubated stationary at the seasonal *in situ* temperature for eight weeks and then frozen until extraction and gas chromatographic analysis as before.

Duplicate zero-time and weathering controls were included for each pure culture. Zero-time controls were frozen immediately after the addition of cells. Weathering controls, which contained heat-killed cells, were incubated with experimental flasks at the seasonal *in situ* temperature.

Utilization Rate of n-[1-^{14}C] Hexadecane by Four Pure Cultures of Hydrocarbon Degrading Bacteria

One milliliter of a 48-hour pure culture of hydrocarbon degrading bacteria was added to 30 ml serum vials containing nine milliliters basal medium and 0.05 ml unautoclaved SLCO. Each vial received 1 µl n-[1-^{14}C] hexadecane (specific activity, 54 mCi/mmole; 98% radiochemically pure; purchased from Amersham Corporation, Arlington Heights, Illinois). After addition of the radioisotope, vials were sealed and incubated stationary at 25°C. The reaction in duplicate vials was terminated after 0, 3, 8, 24, 48 and 72 hours by injection of one drop of concentrated H_2SO_4.

The $^{14}CO_2$ and bacteria in vials were collected using methodology described by Walker and Colwell (1976b). Samples were added to 10 ml of "cocktail" [4 g OMNIFLUOR (New England Nuclear, Boston, Massachusetts) per liter toluene] and assayed using a liquid scintillation counter. Counts per minute were corrected for background and percent efficiency using n-^{14}C-hexadecane as an internal standard.

Duplicate control vials for hexadecane volatilization and adsorption of radioactivity to cells were included for each pure culture at each incubation period. Activity of control vials was subtracted from that of experimental vials.

Growth of pure cultures was determined after 0, 24, 48 and 72 hours in separate vials using enumeration techniques described previously for total aerobic heterotrophic bacteria.

Effects of Oil on Sediment Bacteria

Four 10 ml sediment subsamples were diluted 1:10 in basal medium. Two of the four diluted samples contained 0.5% V/V autoclaved SLCO. The remaining two samples contained no added oil and served as controls. Samples were incubated stationary for eight weeks at the seasonal *in situ* temperature. At four periods during the eight weeks, total aerobic heterotrophic bacteria were enumerated as described previously.

One milliliter of a 48-hour pure culture of aerobic heterotrophic or hydrocarbon degrading bacteria was added to four flasks containing 98.5 ml basal medium. Two of the flasks contained 0.5% V/V autoclaved SLCO. The remaining two flasks contained no added oil and served as controls. After 0, 0.17, 1, 3, 7, 15 and 30 days stationary incubation at 25°C, the total viable cells were enumerated as described previously for total aerobic heterotrophic bacteria.

Sediments collected from four stations (1/II, 3/II, 1/III, 3/III) during spring and from three stations (1/I, 2/I, 3/I) during fall were used to determine the effects of oil on the number and percent hydrocarbon degrading bacteria. Four 10 ml sediment subsamples were diluted 1:10 in basal medium. Two of the diluted samples contained no added oil and served as controls. Samples were incubated stationary for eight weeks at the seasonal *in situ* temperature. At four or five periods during the eight weeks, the number and percent hydrocarbon degrading bacteria were enumerated as described previously.

The t-test statistic was used to evaluate seasonal differences in bacterial populations and differences due to the addition of oil. Linear regression analyses were employed to correlate bacterial populations with available environmental parameters.

RESULTS

Aerobic Heterotrophic and Hydrocarbon Degrading Bacteria

Aerobic heterotrophic bacteria ranged from 4.6×10^4 to 1.3×10^6/ml wet sediment (Figure 10.1 and Table 1, Appendix I). Statistically signi-

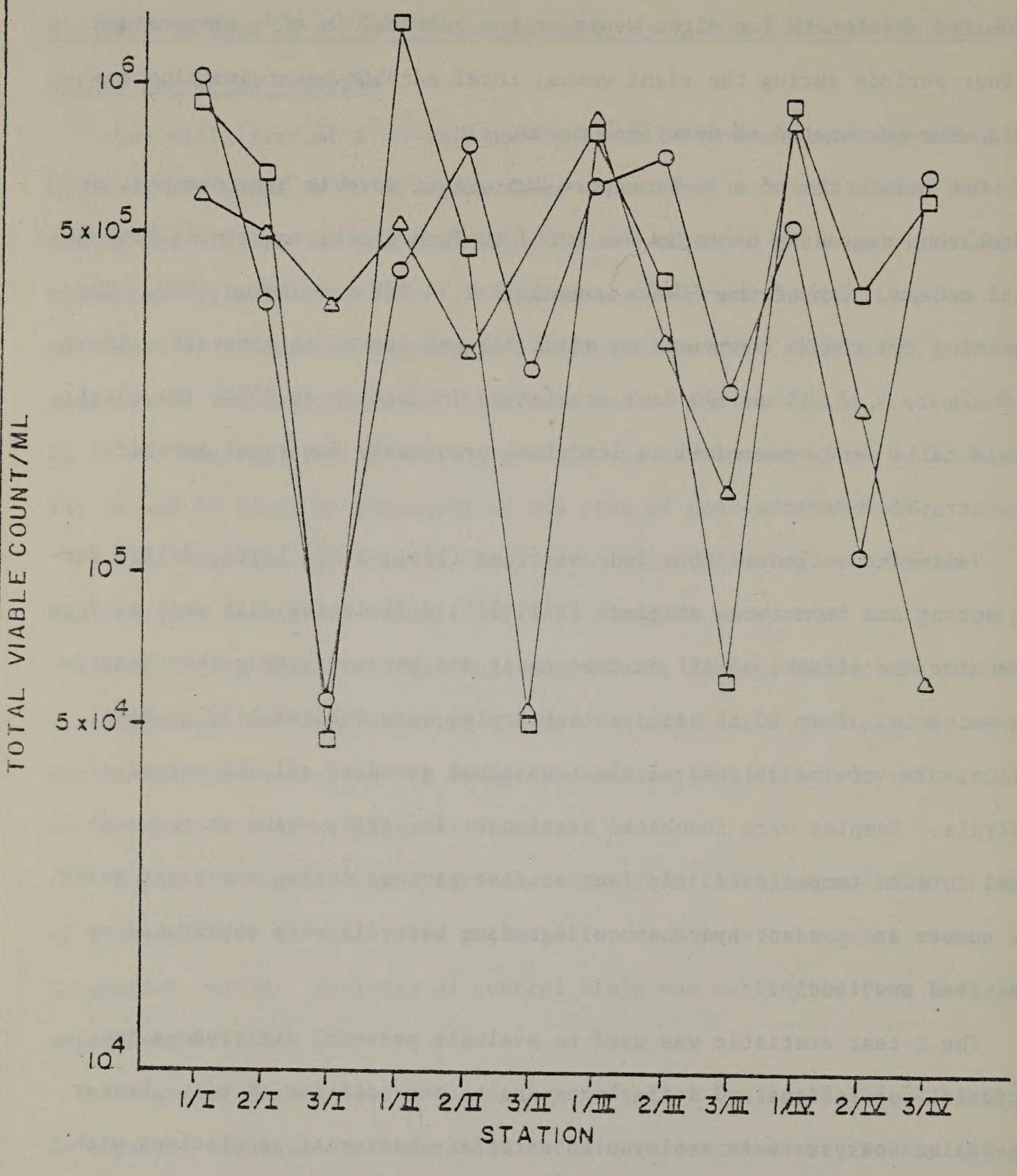

Figure 10.1 Total aerobic heterotrophic bacteria of sediment collected at each station during the winter (Δ), spring (□), and fall (o).

ficant seasonal fluctuations occurred at all stations except 2/I, 2/II, and 3/II. Highest populations did not occur during any one season. Aerobic heterotrophic bacteria decreased as the depth increased ($r = -0.72$, -0.87, -0.55 for winter, spring and fall, respectively).

Hydrocarbon degrading bacteria ranged from 8.0×10^1 to 1.1×10^5/ml wet sediment (Figure 10.2 and Table 2, Appendix I). Seasonal fluctuations occurred at the majority of stations. The significance of seasonal differences, however, could not be determined due to limited replication. Highest populations occurred during fall at the majority of stations. Hydrocarbon degrading bacteria were not highly correlated with depth ($r = -0.49$, -0.56, -0.41 for winter, spring and fall, respectively).

The percent hydrocarbon degrading bacteria ranged from 0.10 to 20.68% (Figure 10.3 and Table 3, Appendix I). Hydrocarbon degrading bacteria were generally less than 1% of the population during winter and spring, but in excess of 1% at the majority of stations during fall. The percent hydrocarbon degrading bacteria was not highly correlated with depth ($r = -0.07$, -0.17, -0.35 for winter, spring and fall, respectively).

A total of 229 aerobic heterotrophic bacteria was selected from Marine Agar 2216 medium. Of these, 88 (38.4%) lost viability on subsequent transfers. Of the remaining 141 pure cultures, 122 were identified to genus level and 16 to family level (Table 10.1). The predominant genera of aerobic heterotrophic bacteria were *Bacillus*, *Vibrio* and *Pseudomonas*. Species of the *Enterobacteriaceae* were also frequently isolated.

A total of 166 bacteria was isolated from growth in MPN oil tubes. Of these, six exhibited growth in fresh oil medium. Five of the six isolated were identified as species of the genus *Pseudomonas*. The remaining pure culture was identified as a species of the genus *Vibrio*.

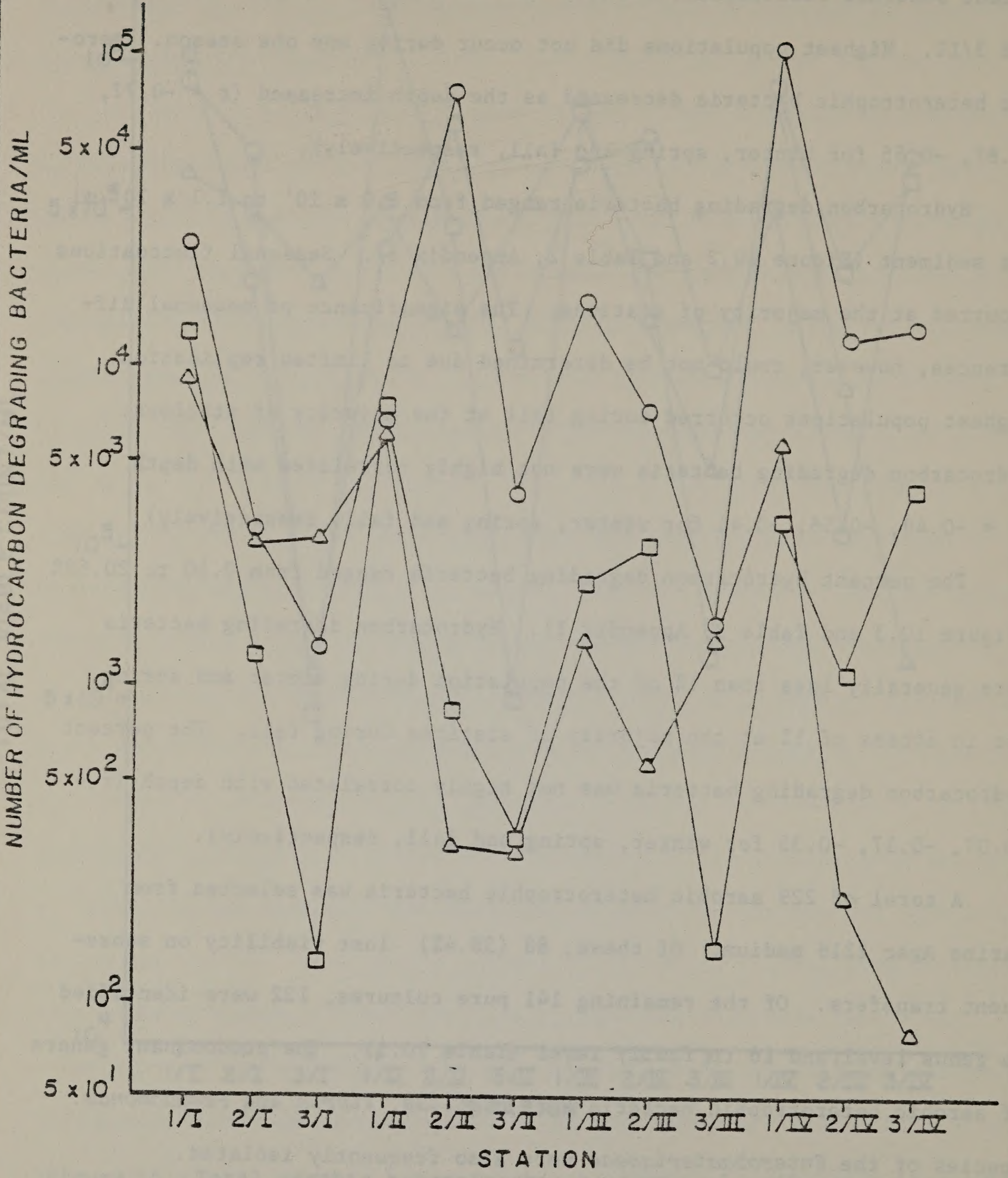

Figure 10.2 Number of hydrocarbon degrading bacteria of sediment collected at each station during the winter (Δ), spring (□), and fall (o).

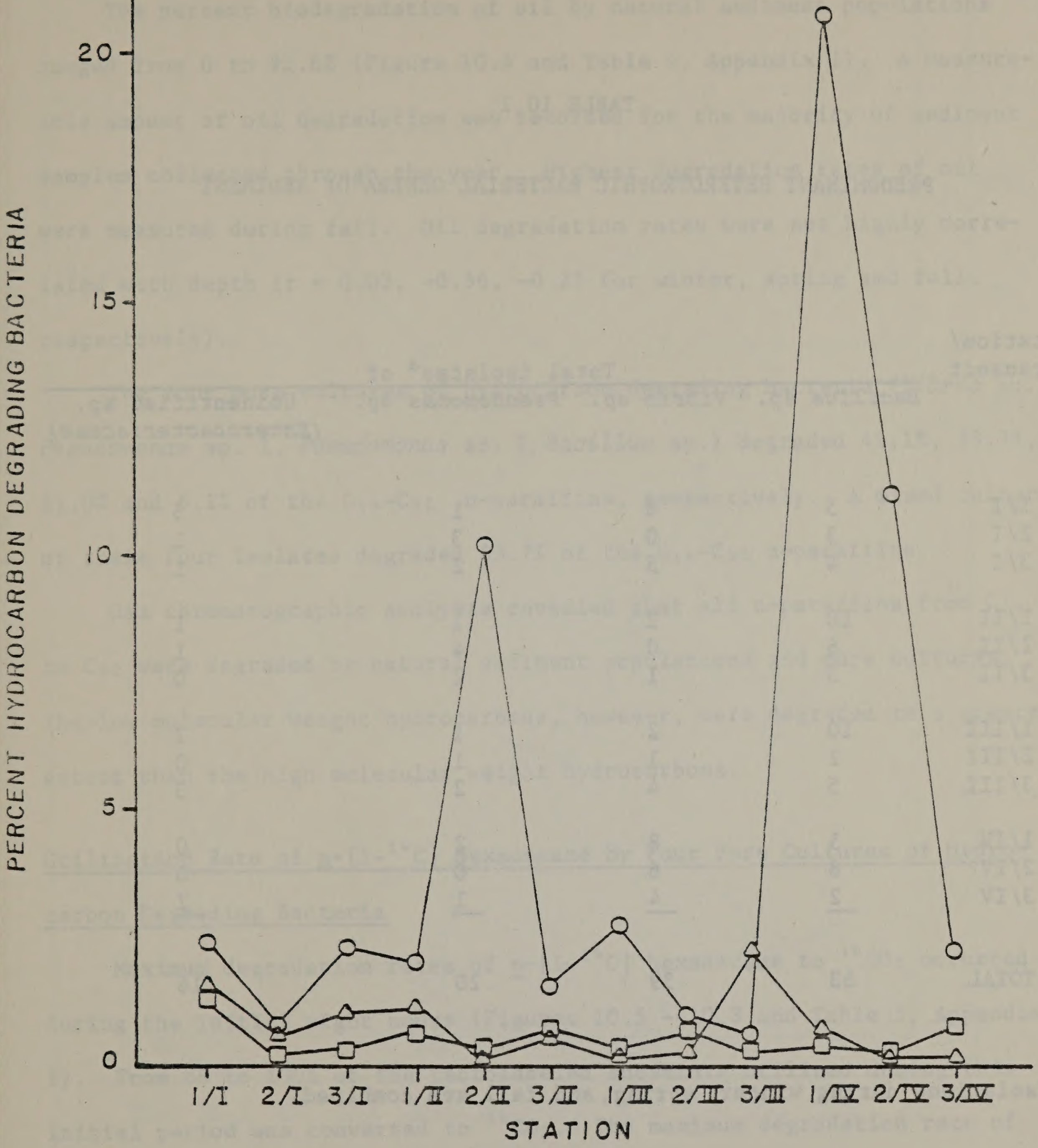

Figure 10.3 Percent hydrocarbon degrading bacteria of sediment collected at each station during the winter (Δ), spring (□), and fall (o).

TABLE 10.1

PREDOMINANT HETEROTROPHIC BACTERIAL GENERA OF SEDIMENT

Station/ Transect	Total isolates[a] of			
	Bacillus sp.	*Vibrio* sp.	*Pseudomonas* sp.	Unidentified sp. (*Enterobacteriaceae*)
1/I	5	6	1	5
2/I	3	0	3	1
3/I	4	5	2	1
1/II	10	2	1	1
2/II	6	0	4	1
3/II	3	1	2	0
1/III	10	2	1	2
2/III	2	1	1	0
3/III	5	4	2	3
1/IV	5	8	2	0
2/IV	8	6	0	0
3/IV	2	4	1	2
TOTAL	63	39	20	16

[a] Isolations during winter, spring, and fall are combined.

Biodegradation of Oil by Sediment Bacteria

The percent biodegradation of oil by natural sediment populations ranged from 0 to 91.6% (Figure 10.4 and Table 4, Appendix I). A measureable amount of oil degradation was recorded for the majority of sediment samples collected through the year. Highest degradation rates of oil were measured during fall. Oil degradation rates were not highly correlated with depth (r = 0.03, -0.56, -0.25 for winter, spring and fall, respectively).

The four pure cultures of hydrocarbon degrading bacteria (*Vibrio* sp., *Pseudomonas* sp. 1, *Pseudomonas* sp. 2, *Bacillus* sp.) degraded 41.1%, 33.3%, 51.0% and 6.1% of the C_{14}-C_{32} n-paraffins, respectively. A mixed culture of these four isolates degraded 83.7% of the C_{14}-C_{32} n-paraffins.

Gas chromatographic analysis revealed that all n-paraffins from C_{14} to C_{32} were degraded by natural sediment populations and pure cultures. The low molecular weight hydrocarbons, however, were degraded to a greater extent than the high molecular weight hydrocarbons.

Utilization Rate of n-[1-^{14}C] Hexadecane by Four Pure Cultures of Hydrocarbon Degrading Bacteria

Maximum degradation rates of n-[1-^{14}C] hexadecane to $^{14}CO_2$ occurred during the initial eight hours (Figures 10.5 - 10.8 and Table 5, Appendix I). From 88 to 100% of the radiolabeled substrate utilized during this initial period was converted to $^{14}CO_2$. The maximum degradation rate of hexadecane to CO_2 was 0.03 µg/day, assuming no significant isotope effect.

The period of maximum uptake of radioactivity by cells varied with the pure culture. Uptake, expressed as percent of the total utilized, however, was greatest after the initial eight hour period.

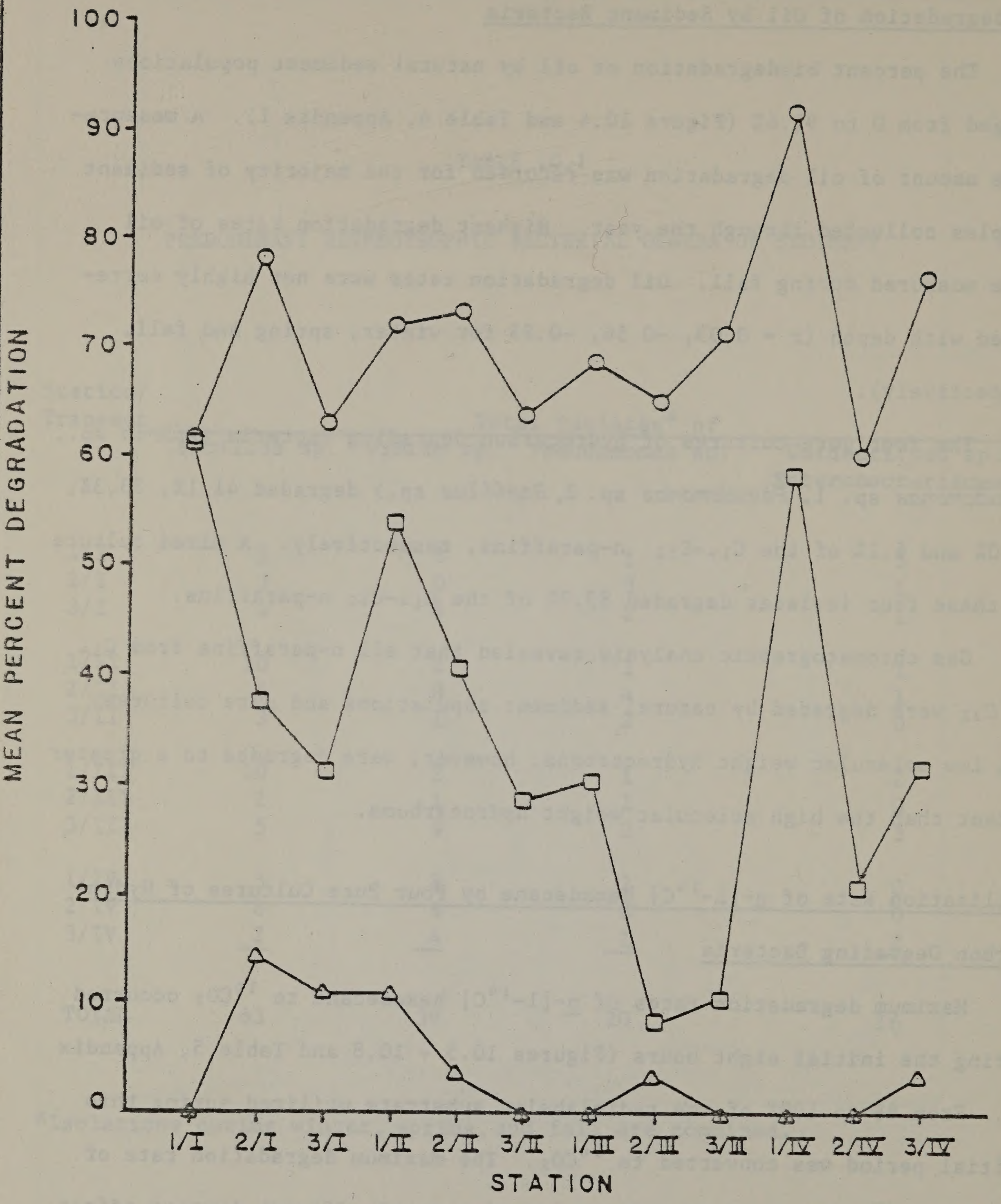

Figure 10.4 Mean percent degradation of South Louisiana Crude Oil by sediment collected at each station during the winter (Δ), spring (□), and fall (o).

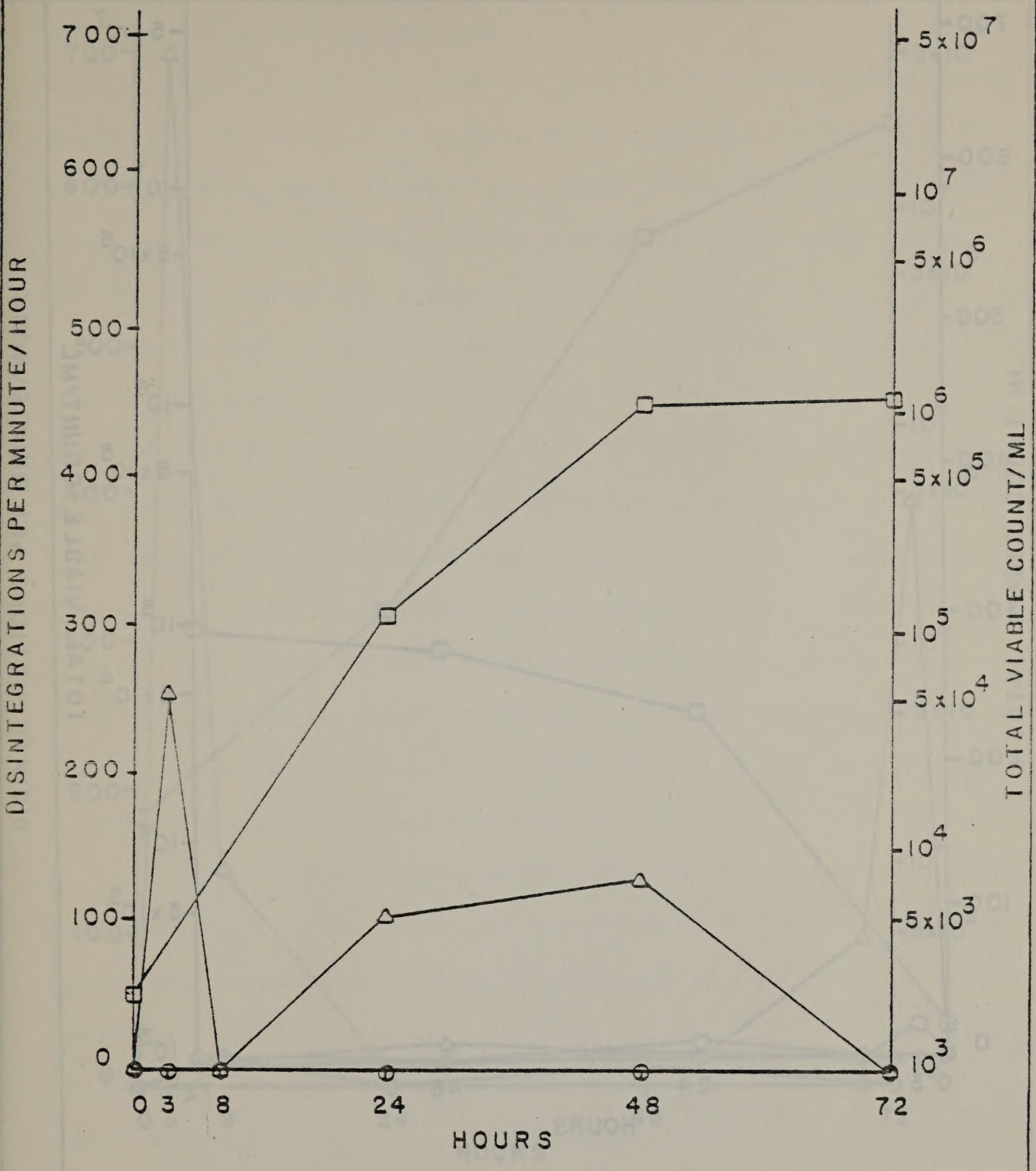

Figure 10.5 Utilization rate of n-[1-^{14}C] hexadecane by *Vibrio* sp. Mineralization to $^{14}CO_2$ (Δ); uptake of radioactivity by cells (o); growth (□).

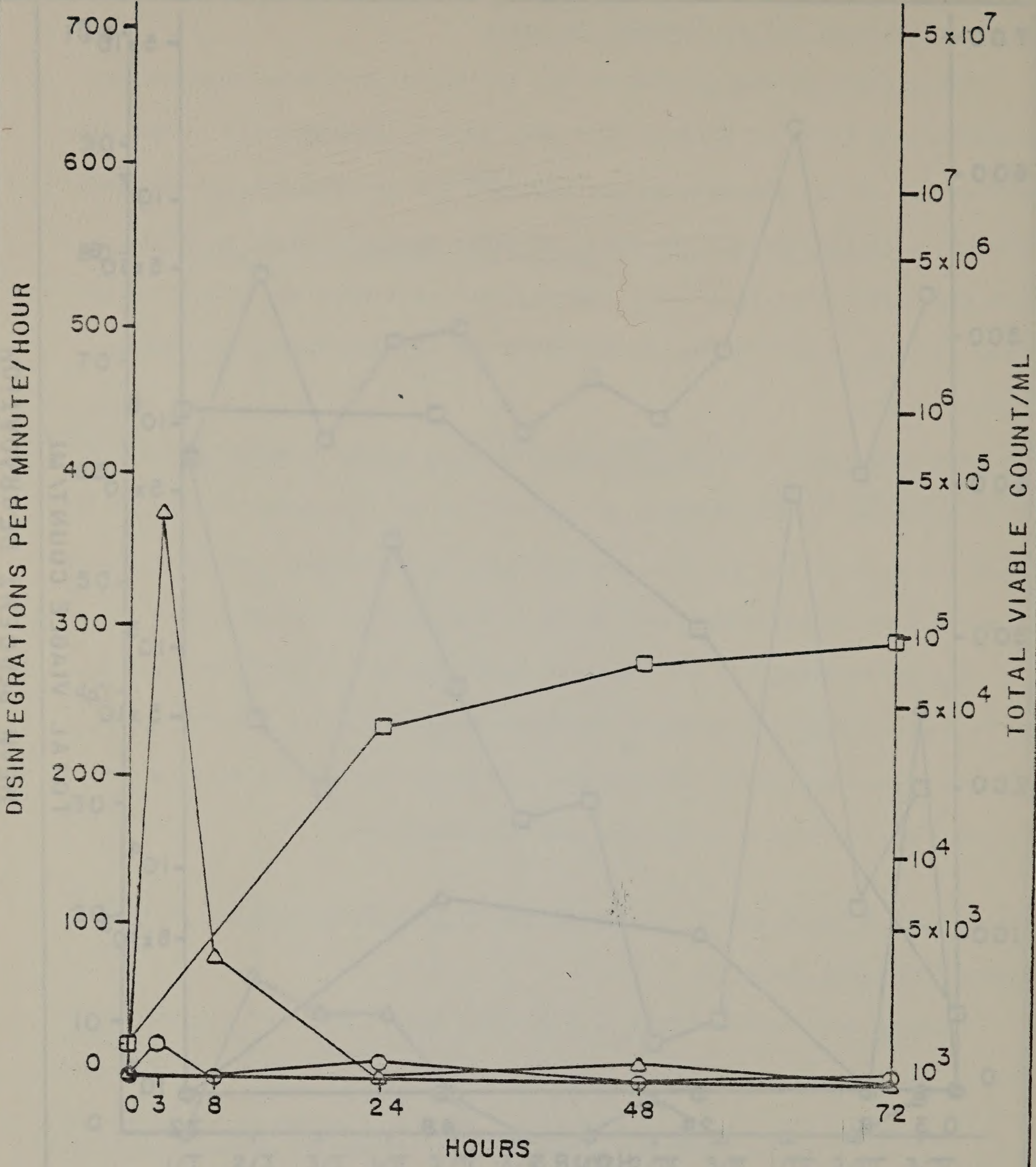

Figure 10.6 Utilization rate of *n*-[1-^{14}C] hexadecane by *Pseudomonas* sp. 1. Mineralization to $^{14}CO_2$ (Δ); uptake of radioactivity by cells (o); growth (□).

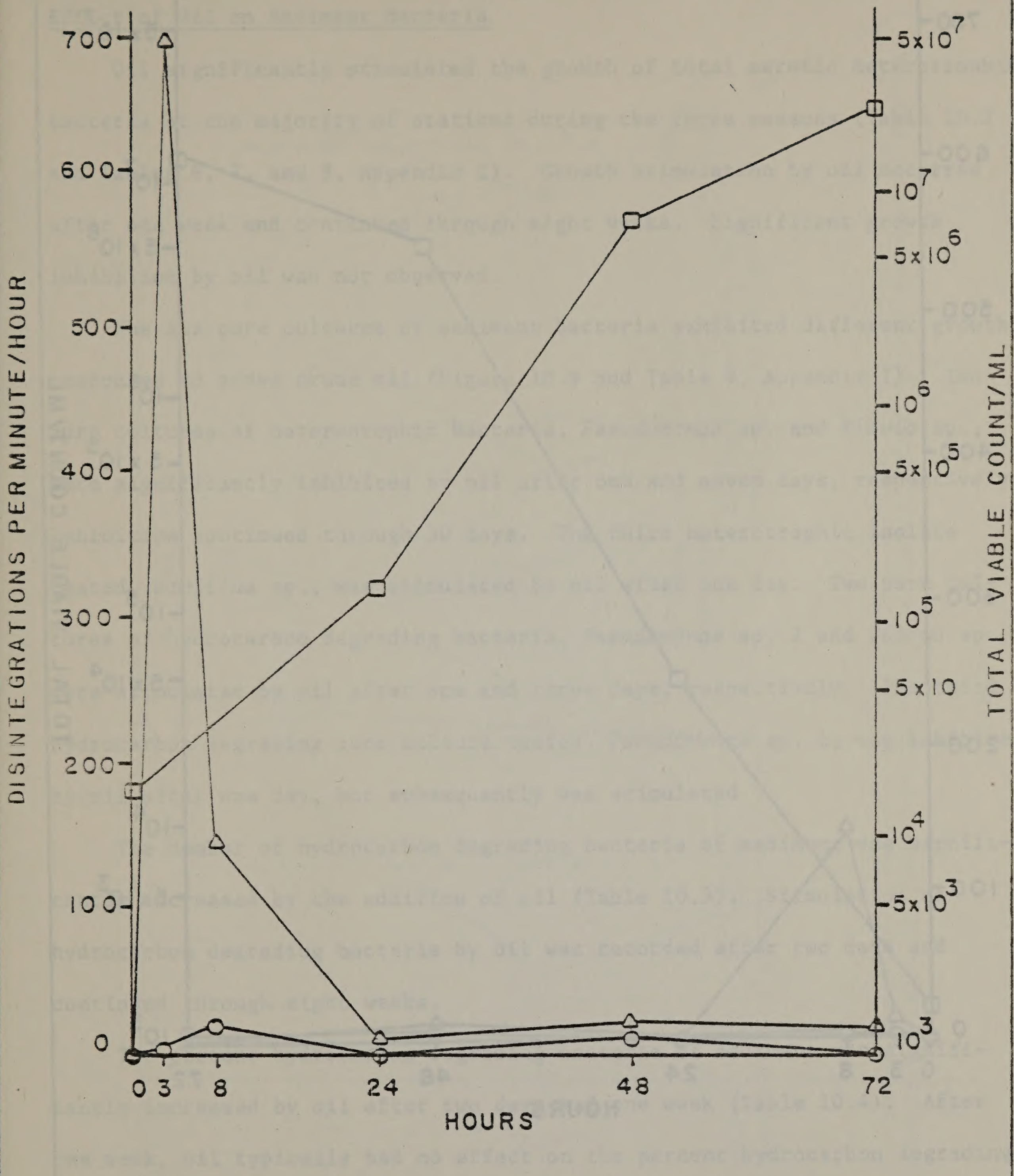

Figure 10.7 Utilization rate of n-[1-^{14}C] hexadecane by *Pseudomonas* sp. 2. Mineralization to $^{14}CO_2$ (Δ); uptake of radioactivity by cells (o); growth (□).

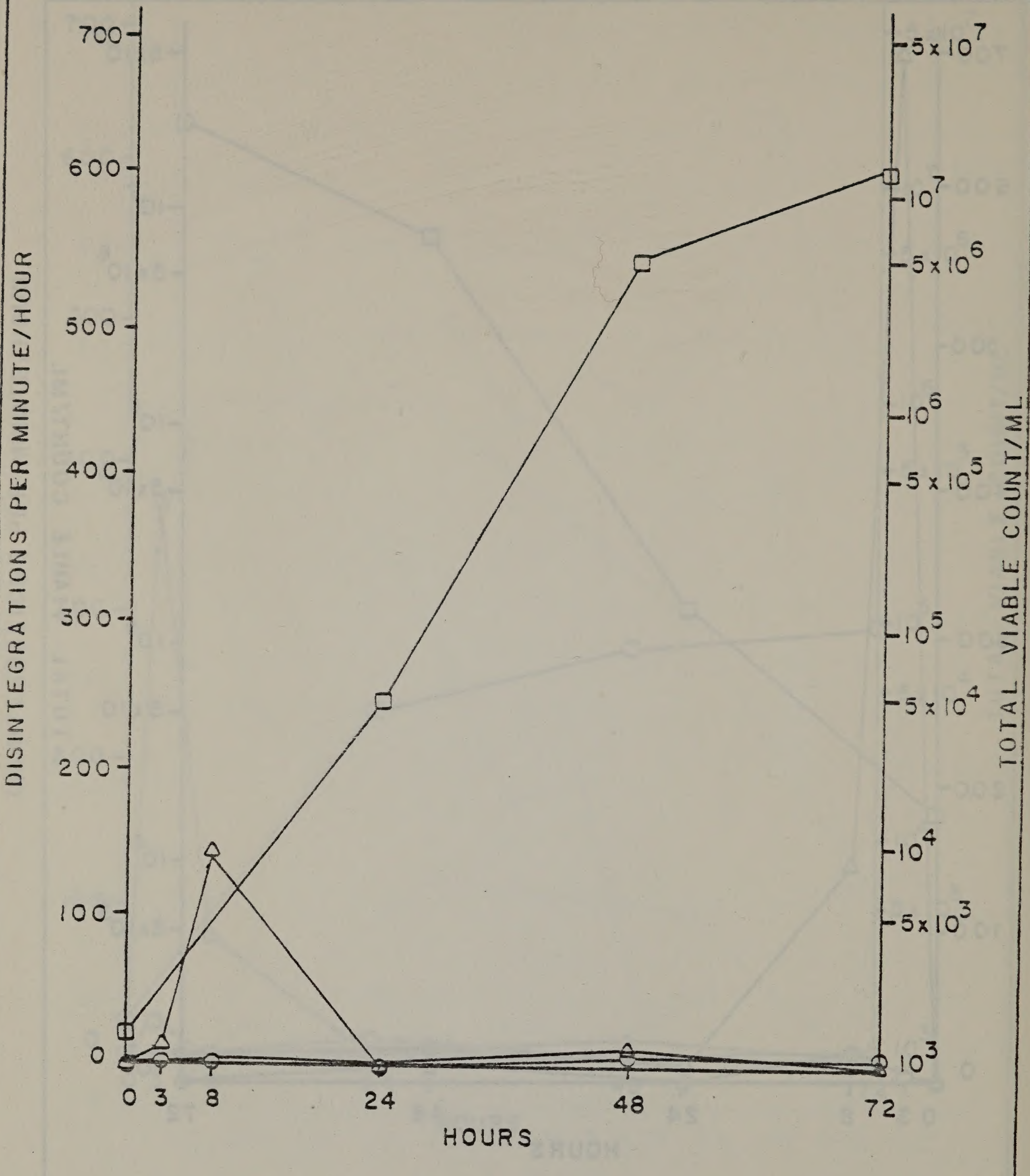

Figure 10.8 Utilization rate of n-[1-^{14}C] hexadecane by *Pseudomonas* sp. 3. Mineralization to $^{14}CO_2$ (Δ); uptake of radioactivity by cells (o); growth (□).

Effect of Oil on Sediment Bacteria

Oil significantly stimulated the growth of total aerobic heterotrophic bacteria at the majority of stations during the three seasons (Table 10.2 and Tables 6, 7, and 8, Appendix I). Growth stimulation by oil occurred after one week and continued through eight weeks. Significant growth inhibition by oil was not observed.

The six pure cultures of sediment bacteria exhibited different growth responses to added crude oil (Figure 10.9 and Table 9, Appendix I). Two pure cultures of heterotrophic bacteria, *Pseudomonas* sp. and *Vibrio* sp., were significantly inhibited by oil after one and seven days, respectively. Inhibition continued through 30 days. The third heterotrophic isolate tested, *Bacillus* sp., was stimulated by oil after one day. Two pure cultures of hydrocarbon degrading bacteria, *Pseudomonas* sp. 2 and *Vibrio* sp., were stimulated by oil after one and three days, respectively. The third hydrocarbon degrading pure culture tested, *Pseudomonas* sp. 1, was inhibited by oil after one day, but subsequently was stimulated.

The number of hydrocarbon degrading bacteria of sediment was significantly increased by the addition of oil (Table 10.3). Stimulation of hydrocarbon degrading bacteria by oil was recorded after two days and continued through eight weeks.

The percent hydrocarbon degrading bacteria of sediment was significantly increased by oil after two days and one week (Table 10.4). After one week, oil typically had no effect on the percent hydrocarbon degrading bacteria (inhibition of percent hydrocarbon degrading bacteria after eight weeks in the spring did not occur in the fall).

DISCUSSION

Enumeration of sediment bacteria during winter, spring and fall indi-

TABLE 10.2

EFFECT OF SOUTH LOUISIANA CRUDE OIL (SLCO) ON THE GROWTH OF AEROBIC HETEROTROPHIC BACTERIA IN SEDIMENT. THE DATA (MEAN ± 1 STANDARD DEVIATION) WERE ANALYZED BY THE t-TEST

Season	Stations Sampled	Incubation Period (weeks)	Number of Stations Where Growth of Bacteria was		
			Stimulated by SLCO[a]	Inhibited by SLCO[a]	Not Effected by SLCO[b]
Winter	12	2	11	0	1
		4	9	0	3
		6	8	0	4
		8	10	0	2
Spring	12	1	9	0	3
		3	11	0	1
		5	10	0	2
		8	12	0	0
Fall	12	1	11	0	1
		3	9	0	3
		5	10	0	2
		8	11	0	1

[a] Significant at $P < .01$

[b] Not significant at $P < .01$

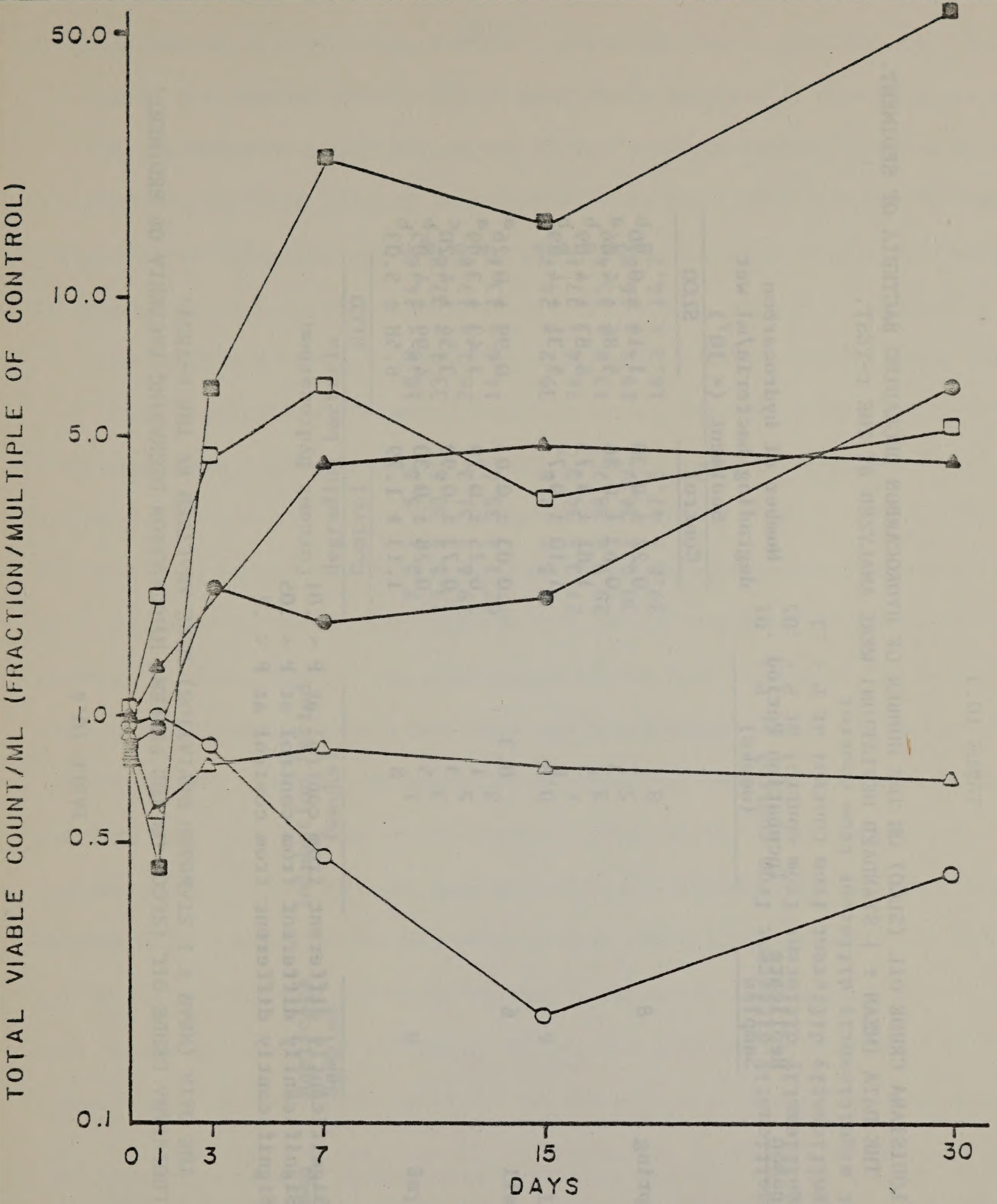

Figure 10.9 Effect of oil on the growth of six pure cultures of sediment bacteria: three aerobic heterotrophic [*Bacillus* sp. (□), *Vibrio* sp. (o), *Pseudomonas* sp. (Δ)] and three hydrocarbon degrading bacteria [*Pseudomonas* sp. 1 (▣), *Vibrio* (●), *Pseudomonas* sp. 2 (▲)].

TABLE 10.3

EFFECT OF SOUTH LOUISIANA CRUDE OIL (SLCO) ON THE NUMBER OF HYDROCARBON DEGRADING BACTERIA OF SEDIMENT. THE DATA (MEAN ± 1 STANDARD DEVIATION) WERE ANALYZED BY THE t-TEST.

Season	Replicate Samples	Incubation Period (weeks)	Number of hydrocarbon degrading bacteria/ml wet Sediment (× 10^7)	
			Control	SLCO
Spring	8	1	0.22 ± 0.30	1.14 ± 0.90[b]
		3	0.63 ± 0.86	5.86 ± 4.66[a]
		5	1.81 ± 3.73	6.83 ± 4.69[b]
		8	1.10 ± 0.75	5.31 ± 4.89[b]
Fall	6	0.3	0.03 ± 0.03	0.99 ± 0.26[a]
		1	0.25 ± 0.29	7.43 ± 3.99[a]
		3	0.73 ± 0.90	3.54 ± 4.20[c]
		5	0.28 ± 0.30	4.99 ± 4.85[b]
		8	1.13 ± 1.79	6.58 ± 5.03[b]

[a] Significantly different from control at P < .01

[b] Significantly different from control at P < .05

[c] Significantly different from control at P < .2

TABLE 10.4

EFFECT OF SOUTH LOUISIANA CRUDE OIL (SLCO) ON THE PERCENT HYDROCARBON DEGRADING BACTERIA OF SEDIMENT. THE DATA (MEAN ± 1 STANDARD DEVIATION) WERE ANALYZED BY THE t-TEST.

Season	Replicate Samples	Incubation Period (weeks)	Percent Hydrocarbon degrading bacteria	
			Control	SLCO
Spring	8	1	7.5 ± 5.5	18.8 ± 14.5[c]
		3	23.5 ± 30.6	33.1 ± 32.8[d]
		5	29.4 ± 31.3	26.1 ± 17.8[d]
		8	62.1 ± 35.4	14.6 ± 13.1[a]
Fall	6	0.3	3.5 ± 2.8	39.2 ± 24.7[a]
		1	27.3 ± 34.3	76.4 ± 27.1[b]
		3	29.9 ± 38.7	13.8 ± 15.4[d]
		5	30.4 ± 34.9	19.4 ± 16.8[d]
		8	39.8 ± 47.6	16.3 ± 14.7[d]

[a] Significantly different from control at P < .01
[b] Significantly different from control at P < .05
[c] Significantly different from control at P < .1
[d] Not significantly different from control

cated that populations are not static, but fluctuate from season to season. The significance of seasonal variations in the number and percent hydrocarbon degrading bacteria could not be determined due to limited replication. Statistically significant fluctuations in total aerobic heterotrophic bacteria were observed at a majority of the stations, but may be only a consequence of spatial variation. Replicate grab samples taken at Stations 1/II, 2/II and 3/II during fall revealed no significant difference between grabs at two of the stations. At Station 3/II, however, total aerobic heterotrophic bacteria of replicate grabs varied approximately tenfold.

Sediment bacterial populations were not evenly distributed over the STOCS. Highest populations of aerobic heterotrophic bacteria occurred at shallow depth stations near terrestrial influences. This distribution of bacteria in the marine environment has been attributed to the higher concentration of organic matter in nearshore sediments (Zobell, 1946).

The percent hydrocarbon degrading bacteria of sediment has been directly correlated with the concentration of contaminating oil (Hood *et al.*, 1975; Walker and Colwell, 1976). For this reason, the percent hydrocarbon degrading bacteria has been proposed as an indicator of oil pollution. A low percent hydrocarbon degrading population (generally < 1%) during winter and spring indicated the pristane nature of the STOCS. However, a higher percent hydrocarbon degrading population (> 1%) occurred during the fall. The observed seasonal variations must be considered if the percent hydrocarbon degrading bacteria is used as an indicator of oil pollution on the STOCS.

Oil biodegradation rates varied seasonally, with highest rates recorded at all stations during fall. Fluctuations in degradation rates may not have been entirely due to season, because of variations in the sterilization and concentration of oil added to degradation flasks. The fall high for degradation rates of oil, however, coincided with a seasonal high in the number and percent hydrocarbon degrading bacteria.

Oil biodegradation rates did not appear to vary geographically, nor did they appear to be related to transect or depth. Likewise, the number and percent hydrocarbon degrading bacteria were not effected by transect or depth.

The addition of oil to sediment increased bacterial populations. Stimulation of aerobic heterotrophic bacteria by oil suggests that it serves as a nutrient source for growth. This contention is supported by two lines of evidence: 1) oil significantly increased the number of hydrocarbon degrading bacteria, and 2) a significant fraction of oil was degraded. Oil not only increased bacterial populations, but also changed the relative abundance of aerobic heterotrophic bacteria. Pure culture studies indicated that certain species of sediment bacteria are stimulated by oil while others are inhibited. Studies with natural sediment populations indicated that oil increased the percent of the bacteria in the population capable of degrading oil. The fact that oil increased the percent hydrocarbon degrading bacteria during the first week only was likely due to substrate limitation after one week in oil-containing flasks.

CONCLUSIONS

1. The data collected here on total aerobic heterotrophic bacteria, and number and percent hydrocarbon degrading bacteria are of limited value because of a lack of sufficient replication to establish significant seasonal and spatial variations, and only one year of data.

2. Oil biodegradation potential of sediment of the STOCS varies seasonally, but is evenly distributed geographically.

3. The addition of oil to sediment increases bacterial populations, but changes the composition of the population to one more capable of degrading oil.

LITERATURE CITED

Atlas, R. M., and R. Bartha. 1972a. Biodegradation of petroleum in seawater at low temperatures. Can. J. Microbiol. 18:1851-1855.

____________. 1972b. Degradation and mineralization of petroleum in seawater: Limitation by nitrogen and phosphorus. Biotechnol. Bioeng. 14:309-318.

Bergey's Manual of Determinative Bacteriology. 1974. Williams and Wilkins Co., Baltimore, 8th Edition.

Butler, J. N., B. F. Morris and T. D. Sleeter. 1976. The fate of petroleum in the open ocean, pp. 287-297. *In* Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment. American Inst. of Biol. Sci.

Floodgate, G. D. 1972. Biodegradation of hydrocarbons in the sea, pp. 153-171. *In* R. Mitchell (ed.), Water pollution microbiology. Wiley-Interscience, New York.

Giam, C. S. 1978. Heavy molecular weight hydrocarbons in macroepifauna and macronekton, Chapter 5. *In* Environmental studies, south Texas outer continental shelf, biology and chemistry. 1977. Final report to the Bureau of Land Management, Washington, D. C. Contract AA550-CT7-11.

Grossling, B. F. 1976. An estimate of the amounts of oil entering the oceans, pp 5-36. *In* Sources, effects and sinks of hydrocarbons in the aquatic environment. American Inst. of Biol. Sci.

Gunkel, W. 1973. Distribution and abundance of oil oxidizing bacteria in the North Sea, pp 127-139. *In* D. G. Ahearn and S. P. Meyers (ed.), The microbial degradation of oil pollutants. Center for Wetland Resources Publication. No. LSU-SG-73-01, Baton Rouge.

Hodson, R. E., F. Azam, and R. F. Lee. 1977. Effects of four oils on marine bacterial populations: controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:119-126.

Hood, M. A., W. S. Bishop, Jr., F. W. Bishop, S. P. Meyers, and T. Whelan III. 1975. Microbial indicators of oil-rich salt marsh sediments. Appl. Microbial. 30:982-987.

Klein, A. E., and N. Pilpel. 1974. The effects of artificial sunlight upon floating oils. Water Res. 8:79-83.

Lee, R. F. 1976. Metabolism of petroleum hydrocarbons in marine sediments, pp 333-344. *In* Sources, effects and sinks of hydrocarbons in the aquatic environment. American Inst. of Biol. Sci.

________, and C. Ryan. 1976. Biodegradation of petroleum hydrocarbons by marine microbes, pp 119-125. *In* J. M. Sharpley and A. M. Kaplan (eds.) Proceedings of the Third International Biodegradation Symposium. Applied Scince Publishers, London.

McAuliffe, C. D. 1976. Surveillance of the marine environment for hydrocarbons. Mar. Sci. Communications. 2:13-42.

Mechalas, B. J., T. J. Meyers, and R. L. Kolpack. 1973. Microbial decomposition patterns using crude oil, pp 67-79. *In* D. G. Ahearn and S. P. Meyers (eds.) The microbial degradation of oil pollutants. Center for Wetland Resources Publication No. LSU-SG-73-01, Baton Rouge.

Nelson-Smith, A. 1970. The problem of oil pollution of the sea, pp 215-306. *In* F. S. Russell and M. Younge (eds.) Advances in marine biology, Vol. B. Academic Press, New York.

Sackett, W. M., J. M. Brooks, and B. B. Bernard. 1976. Low-molecular-weight hydrocarbons, nutrients, and dissolved oxygen. *In* Environmental studies, south Texas outer continental shelf, biology and chemistry. Third and Fourth Quarterly Reports to the Bureau of Land Management, Washington, D. C. Contract AA550-CT6-17

Soli, G. 1973. Marine hydrocarbonoclastic bacteria: types and range of oil degradation, pp 141-146. *In* D. G. Ahearn and S. P. Meyers (eds.) The microbial degradation of oil pollutants. Center for Wetland Resources Publication No. LSU-SG-73-01, Baton Rouge.

Walker, J. D., P. A. Seesman, and R. R. Colwell. 1974. Effects of petroleum on estuarine bacteria. Mar. Poll. Bull. 5:186-188.

________, and R. R. Colwell. 1976a. Enumeration of petroleum-degrading microorganisms. Appl. Environ. Microbiol. 31:198-207.

____________. 1976b. Measuring the potential activity of hydrocarbon-degrading bacteria. Appl. Environ. Microbiol. 31:189-197.

____________. 1976c. Petroleum: degradation by estuarine microorganisms. pp 197-204. *In* J. M. Sharpley and A. M. Kaplan (eds.) Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, London.

Wardley Smith, J. 1976. Oil spills from tankers, pp 473-481. *In* J. M. Baker (ed.) Marine ecology and oil pollution. John Wiley and Sons, New York.

Zobell, C. E. 1946. Marine Microbiology. Chronica Botanica Co., Waltham.

________. 1969. Microbial modification of crude oil in the sea, pp 317-326. *In* Proceedings of the API/FWPCA Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, New York.

________. 1973. Microbial degradation of oil: Present status, problems, and perspectives, pp 3-16. *In* D. G. Ahearn and S. P. Meyers (eds.) The microbial degradation of oil pollutants. Center for Wetland Resources Publication No. LSU-SG-73-01, Baton Rouge.

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